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# Secondary sexual characters in crawling water beetles (Coleoptera: Haliplidae): evidence for sexual conflict?

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## Abstract

Conflict between sexes occurs as a result of asymmetric reproductive trade-offs during mating. When the rates of these trade-offs differ, sexual conflict can occur, this can lead to coevolutionary arms races between the sexes. Such arms races may result in the evolution of secondary sexual characteristics and the coevolution of sex related structures. This study analysed the morphology of three genera of Haliplidae: *Haliphus*, *Brychius* and *Algophilus* using Scanning Electron Microscopy to determine whether there was evidence of sexual conflict. Males in this family possess small tarsal suckers on the fore and mid-legs that aid the male to grasp the female's dorsal surface during mating, potentially increasing reproductive success. Females also possess micropunctuation on the dorsal surface which may deter male attachment. This study investigates the notion of female evolution of sex related counteradaptations that reduce potentially costly copulations. Results from this study indicate that there is little covariance in secondary sexual characters between the sexes this family as a whole ( $P = 0.934$ ,  $r = -0.030$ ). However, there was significant evidence of differentiation in sex-specific characters amongst certain species, inferring that characters may be evolving phylogenetically. Further investigation into specific clades of Haliplidae would be beneficial, as an equivalent comparison can be made between sexes of the same clade.

Keywords: coevolution; counteradaptation; evolutionary arms races; morphology; sexual arms race; sexual dimorphism.

## Introduction

Darwin developed the theory of sexual selection in 'The Descent of Man and Selection in Relation to Sex' (1871). In this book, Darwin proposed that sexual selection was an advantage which certain individuals possessed over others of the same species or sex that were exclusively beneficial to reproduction (Darwin, 1871). Sexual selection occurs through one of two ways: either intrasexual or intersexual. Intrasexual selection is male-male competition for mates, usually through the evolution of male weapons or direct intra-male competition (Parker, 1979). Intersexual selection occurs through female choice, whereby the female has a preference over which male she will accept for copulation. This enables the female to choose the best quality male (Hostedde & Alarie, 2006). Males that are better-armed or more attractive will obtain the most copulations (Darwin, 1871), consequently maximising the success of their progeny.

When the evolutionary interests of males and females differ (regarding optimal fitness over reproduction), sexual conflict can occur (Parker, 1979; Parker, 2006). Contrasting fitness optimums are triggered by differential investment by males and females during reproduction, normally predicted by anisogamy (differing investment in dimorphic gametes) (Chapman, 2006; Ghiselin, 2010). Conflict of this kind can occur in relation to pre-copulatory competition between the sexes, such as competition over fertilisation and courtship (Chapman, 2006). For example, males often benefit from multiple copulations (Bateman's principle), increasing their reproductive success and the number of progeny sired with each copulation. However, the latter is untrue for females: multiple copulations are often detrimental to females, significantly reducing fitness (Bateman, 1948; Reinhardt *et al*, 2003). Mating is adaptive for males as the production of sperm is less costly than the formation of ova (Yasui, 1997). Consequently, male and female interests rarely coincide, and when one sex evolves a trait that reduces or hinders the fitness of the other sex, antagonistic coevolutionary arms races may occur (Brown *et al*, 2011; Chapman *et al*, 2003). Evolutionary arms races begin when a male evolves a character that increases the cost of mating for females, in turn, lowering the her fitness. (Ronn *et al*, 2006). The costs to female fitness from mating conflicts with males are predicted to drive sexually antagonistic coevolution of the sexes (Gosden & Svensson, 2007). Reproductive conflicts like this link to the chase-away hypothesis which suggests that sexual conflict promotes sexually antagonistic coevolution, rather than a mutualistic coevolution (Pizzari & Snook, 2003).

Coevolutionary arms races exist when one sex acquires a copulatory advantage due to an amplification of a genotypic or phenotypic trait, that drives counteradaptations in the one sex that contest adapted traits in the opposite sex (Arnqvist & Rowe, 2002). For instance, prolonged matings can often cause a fitness cost to females. As a result, associated costs females would be expected in order to resist matings, whilst males would be expected to force copulation as they usually benefit from each copulation. Females often evolve counteradaptations to resist matings that are potentially costly. As a result, an evolutionary arms race of adaptations and counteradaptations occur that can drive the evolution of antagonistic dimorphism's (Red Queen hypothesis) between the sexes (Miller, 2003).

Coevolutionary models predict that conflict between sexes over control during copulation can precipitate the adaptation and coevolution of prehensile secondary sexual structures (Hostedde & Alarie, 2006; Arnqvist & Rowe, 1995). Prehensile structures are appendages or organs adapted for grasping onto females during copulation, preventing female escape and interference from competing males (Darwin, 1871). Adaptive secondary structures like this are known as sexual dimorphism's and can often be the result of sex specific selection, causing the evolution of differing morphological traits between sexes (Hostedde & Alarie, 2006). When the reproductive interests of male and females are not symmetrical one sex (usually the female) obtains a cost related to reproduction and in turn, reduced fitness (Arnqvist & Rowe, 1995; Arbuthnott *et al*, 2013). The female evolves a counteradaptation to contest the adaptation that is detrimental to her fitness. This differentiation of costs and benefits between the sexes is where sexual conflict occurs (Parker, 1979; Benvenuto & Weeks, 2012).

Various male insects have evolved prehensile structures that aid grasping ability during copulation, such as modified tarsi, femora and antennae. Arnqvist & Rowe (1995) studied the coevolution between the sexes in water striders (Hemiptera: Gerridae). Females of this family have evolved counteradaptations in response to male secondary sexual characteristics (sex-specific conflict traits). During the mating season males ambush females, attempting to grasp them with modified abdominal claspers and genitalia, while females try to dislodge males with rapid movements (Gagnon & Turgeon 2011). Some females have evolved counteradaptations in the form modified abdominal spines that reduce male grasping ability

(Arnqvist & Rowe, 1995). Sexual aggravation by males can be detrimental to female fitness as it often results in increased energy expenditure and predation risk. Evolving counteradaptations that contest copulations decreases the risk of aggravation and costs from males (Han *et al*, 2010). Sexual conflict is more likely to lead to sexually antagonistic coevolution when there are significant differences in the fitness optima for conflict traits between the sexes (Chapman, 2006).

Females can still gain benefits from copulations that incur fitness costs providing the male has a high level of fitness and female fecundity increases (presuming the costs are not excessively deleterious) (Parker, 1979). Where no direct gain can be detected, as seen in post-copulatory mate guarding, indirect benefits can be gained through genetic benefits (Yasui, 1997; Chapman, 2006). Hence, progeny of the pair will inherit a proportion of the advantageous traits, this in turn, increases female reproductive success. It is in the male's best interest to minimise costs to his mate, if the costs are inherited by their progeny then his reproductive success decreases. When the costs over reproduction differ, often for the female, sexual conflict occurs, this can lead to females evolving counteradaptations to contest costly copulations from males (Parker, 1979). Arnqvist & Rowe (2002) similarly investigated the coevolution of armament of the sexes and the outcome of sexually antagonistic interactions in 15 water strider species. This study revealed that there was strong overt sexual conflict over copulation rate, due to a lack of equality over reproductive processes between the sexes. Arnqvist & Rowe (1995) stated that copulation in this family almost always commenced with a violent pre-copulatory struggle. Female counteradaptations thereby reduce male prehension and affliction to the female. The coevolution of these sexual structures underpins the driving force of sexual conflict and evolutionary arms races between the sexes (Arnqvist & Rowe, 2002; 1995). Arnqvist & Rowe argue that premating struggles in insects encourage female choice for males with 'good genes', as female abdominal spines promote adaptive female mate choice.

Sexual conflict occurs in many species of water beetles, most commonly in the form of sexual dimorphism, where male and female form differ in morphological traits. Bilton *et al* (2008) studied the sexual dimorphism between the sexes of *Hydroporus memnonius* (Coleoptera: Dytiscidae). Secondary sexual characteristics were identified in both sexes and analysed using Scanning Electron Microscopy (SEM). This study analysed female pronotal and elytral micro-

sculpture, evidence indicated that there were two different morphologies of females: smooth and matt morphs. The two morphs differ in density and intensity of micro-reticulation. Males in this species possess modified adhesive suckers (setae) (a conflict trait) on the ventral side of the second, third and fourth tarsomeres of the fore-tarsi, which are reinforced by flexible rods that are likely to have evolved from a bundle of fused setae (Gorb, 2001).

During copulation, dytiscid males grasp onto the females pronotum or the anterior part of the elytra with their fore and mid-legs; these suckers are expected to significantly aid attachment (Gorb, 2001). Whilst the male grasps the female, she aggressively tries to detach the male through rigorous shaking (Green *et al.*, 2013). Bilton *et al* (2008) proposed that males with increased numbers of suckers were likely to be more successful in grasping onto both morphs of female during copulation. It would be expected that males with well developed setae would obtain more mating from females as they are better able to adhere to her dorsal surface (Bilton *et al*, 2008). Bergsten *et al* (2001) similarly studied the adhesive suckers of three species of dytiscid (*Dytiscus dimidiatus*, *Dytiscus lapponicus* and *Graphoderus zonatus*) and found that their observations were consistent with the theories of evolutionary arms races and female counteradaptations. This type of sexual conflict is considered as antagonistic as the male forcefully grasps the female, leaving her with little choice over copulation. If he is successful, the female often cannot release from his grasp.

Green *et al* (2013) studied this form of antagonistic coevolution between the sexes similarly to Bilton *et al* (2008) and Bergsten *et al*, (2001). This study used biomechanics to examine the male setae in *D. lapponicus* and *G. zonatus* and their adhesive performance in attaching to smooth and rough morphs of females. They found that the adhesive force on the rough surface was lower than in the smooth morph. This evidence supports sexual conflict theory in this family of diving beetles, as the counteradaptation of the rough dorsal surface (micro reticulation) in matt females corresponds to antagonistic sexual conflict theory (Green *et al*, 2013).

SEM has been widely used to study secondary sexual structures that cause sexual conflict. Wolfe & Zimmerman (1984) studied the micro-punctuation of the elytral and pronotal surfaces in Dytiscidae using SEM. Results from this study suggest that there are different

forms of reticulation and punctation present on the elytra and pronotum, particularly in females. Prehensile structures have been described and analysed using SEM in the males of dytiscid beetles (Bilton *et al*, 2007; Bergsten *et al*, 2001). Stork (1980) also used SEM to examine the adhesive setae of 84 different species of Coleoptera. Morphology of the adhesive setae were described to have two well defined regions: the shaft and the distal plate (Stork, 1980).

This study examines sexual dimorphism in the crawling water beetle family Coleoptera: Haliplidae, which can be divided into 5 sub genera: *Haliphus*, *Brychius*, *Peltodytes*, *Algophilus* and *Apteraliphus* (Vondel & Dettner, 1997). We investigate possible evidence for sexual conflict in three genera; *Haliphus*, *Brychius* and *Algophilus* (Table 1). This family can be located in a number of habitats, largely freshwater and brackish water (Vondel & Dettner, 1997; Krell, 2008). Morphological observations of Haliplidae suggest sexual dimorphism between the sexes, through male tarsal morphology and female dorsal punctuation. Similarly to Dytiscidae, copulation begins by the male approaching the female, grasping her dorsal surface with his fore and mid-legs. Adhesion is aided by small suckers on the second and third tarsal segments. It is expected that during copulation, the male targets his suckers on the pronotum and the anterior region of the elytra. The females have punctures (micro punctation) along the pronotal and elytral discs that reduce the adherent potential for males to grasp onto them. There is often a struggle from the female to resist the male, thus the presence of setae on the male tarsi may important in aiding male adherence to the female dorsal surface and increasing copulatory success. Structural modifications like this, which are seen in Dytiscidae are considered to be driven by sexual conflict and are a result of an antagonistic co-evolution between the sexes (Hostedde & Alarie, 2006).

In the present study, scanning electron microscopy is used to quantify differences in micro sculpture between the male and female form of Haliplidae, and possible evidence for sexual conflict is examined. Sexual conflict has as be documented in Dytiscidae which are a closely related group in the aquatic Adephaga (Dressler & Beutel, 2010). There is evidence of a coevolution within Dytiscidae, which merits examination of the closely related dimorphic traits that are seen in Haliplidae. This study investigates the differentiation in morphology between species and possible covariance in sex-specific traits between male secondary

structures and female sculpture. This is the first study to look at possible evidence for sexual dimorphism and sexual conflict in Haliplidae.

### Aims and objectives

- I. Use SEM to investigate whether Haliplidae males have modified suckers.
- II. Use SEM to investigate whether Haliplidae females have modified dorsal sculpture
- III. Investigate whether species differ in these characters and if there is evidence of male and female coevolution between three subgenera of Haliplidae (*Haliplus*, *Brychius* and *Algophilus*).

### Material and methods

#### Sample collection and preparation

Specimens of Haliplidae were collected using a D-framed pond net (30x25cm; 1mm mesh) from a number of localities in Europe and South Africa (Table 1). Beetles were killed with ethyl acetate vapour, and preserved in 70% ethanol until required for electron microscopy.

**Table 1.** Specimens of Haliplidae studied.

Order	Coleoptera		
Family	Haliplidae		
Genus	<i>Haliplus</i> <i>Brychius</i> <i>Algophilus</i>		
Species	No. individuals ♂   ♀		Locality
<i>Algophilus lathridioides</i>	2	1	Berg River floodplain, South Africa
<i>Brychius elevatus</i>	1	1	Ayrshire, United Kingdom
<i>Haliplus africanus</i>	3	3	Bredasdorp, South Africa
<i>Haliplus flavicollis</i>	2	2	Berezina River, Belarus
<i>Haliplus immaculatus</i>	4	3	Cross Drain, Lincolnshire, UK
<i>Haliplus laminatus</i>	2	2	Cross Drain, Lincolnshire, UK
<i>Haliplus lineatocollis</i>	4	3	Rio Mundo, Albacete, Spain
<i>Haliplus mucronatus</i>	2	3	Rio Mundo, Albacete, Spain
<i>Haliplus obliquus</i>	1	3	Cross Drain, Lincolnshire, UK



Total number of specimens: 50

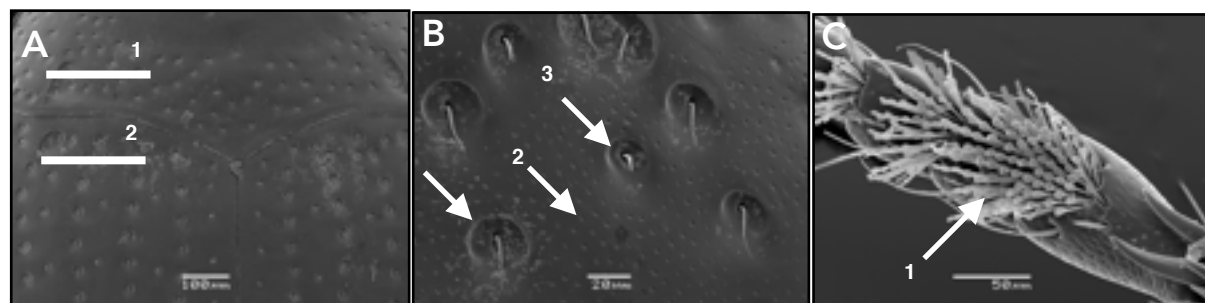
All specimens were collected by D. T. Bilton.

### Specimen preparation

Beetles were removed from 70% ethanol and sexed by presence of setae on fore and mid-legs of males (Figure 1c) using a low powered microscope (Leica EZ4). They were then placed in a drying oven for three days to ensure internal moisture content was minimal. Fore and mid-legs were removed from the right hand side of the beetle using fine forceps, ventral side uppermost. If there was damage to the tarsi then the legs were removed from the opposite side. Samples were mounted dorsal side uppermost with the fore and mid-legs placed to the left of the beetle. The legs were mounted with the underside of the tarsi facing uppermost; care was taken whilst orientating the legs to expose the setae for examination under the microscope. All specimens were then layered in a gold coating using an Emitech K550 sputter coating unit.

### Electron microscopy

Samples were examined using JEOL JSM5600LV scanning electron microscope. Micrographs were taken of female pronotal and elytral surface at x150. Two micrographs were taken horizontally across the pronotal disc and two photos across the elytral disc, all at x700 (Figure 1A). Male fore and mid-legs were photographed at x190, tarsi were photographed twice at x500 to obtain micrographs of setae (Figure 1c) and a close up of the setae plates at x2700. Female tarsi were photographed but only one female per species to allow comparison to male tarsal structures; female legs were not studied quantitatively.



**Figure 1.** Micrograph of male and female structures in *Haliphus ruficollis*. A: Dorsal surface, 1: Indicates the study area on the pronotal disc and 2: indicates the study area on the elytral disc during analysis. B: Elytral disc 1: a large pore, 3: medium pore and 2: small pore. C: Male tarsomere, 1: indicates the tarsal suckers. Scale bars are given bottom middle of each micrograph.

## Image analysis and specimen measurements

All images were analysed using ImageJ 10.2 image analysis software. Male setae were counted on fore and mid-legs, and five setal diameters were measured (measured across the midline of the suction plate). Total female pore density of the pronotum and elytra was calculated, and densities of small, medium and large pores were recorded. Diameters of four large pores were measured (across the midline of the pore).

## Statistical analysis

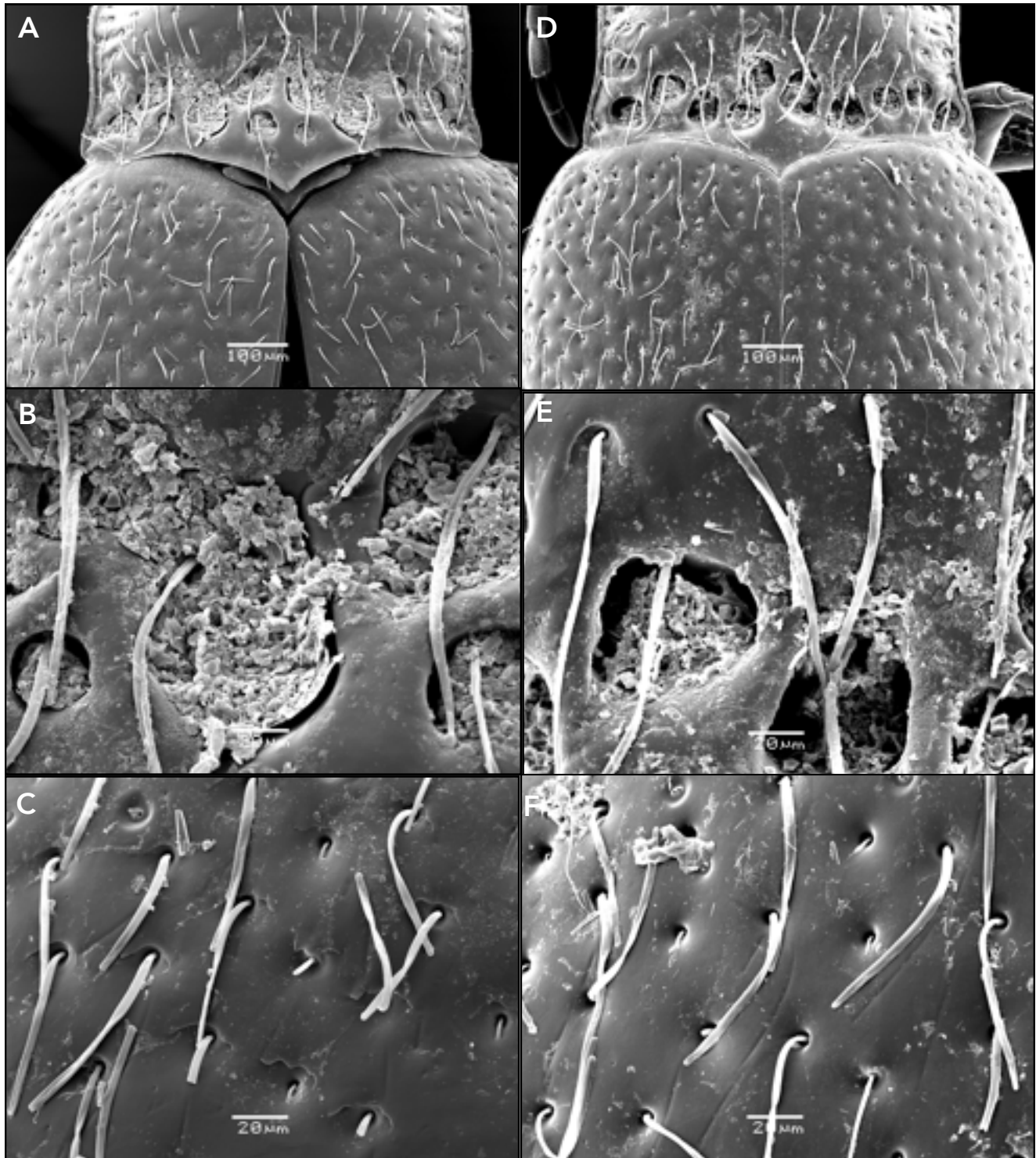
Minitab 16, IBM SSPS 2.0 and Primer 6 software were used for all statistical analyses. Ten species were analysed using SEM, however, due to small sample size certain species were exempt from statistical analysis as to not break the assumptions of Analysis of variance (ANOVA) (Table 2). Anderson-Darling normality tests were carried out for all data. Where the data did not conform to a normal distribution, two methods were used to normalise the data. Firstly, tests for equal variances were adopted to determine homogeneity of variances. Secondly, where the variances were not homogeneous double log transformations were applied to the data to transform it to a normal distribution. One-way ANOVA and Kruskal-Wallis tests were employed to investigate differences in the dorsal surface between the different female species. Kruskal-Wallis tests were adopted for analysis of non-parametric data and one-way ANOVA were adopted for parametric data. Post-hoc analysis were carried out after all significant ANOVAS by Tukey tests to determine differing species. Permutational MANOVA (PERMANOVA) was used to further test for differences in characters to investigate differences in overall in male specific traits. Possible associations between male and female sex-specific characters were analysed using spearman's rank correlation coefficient.

**Table 2.** Species used in analysis.

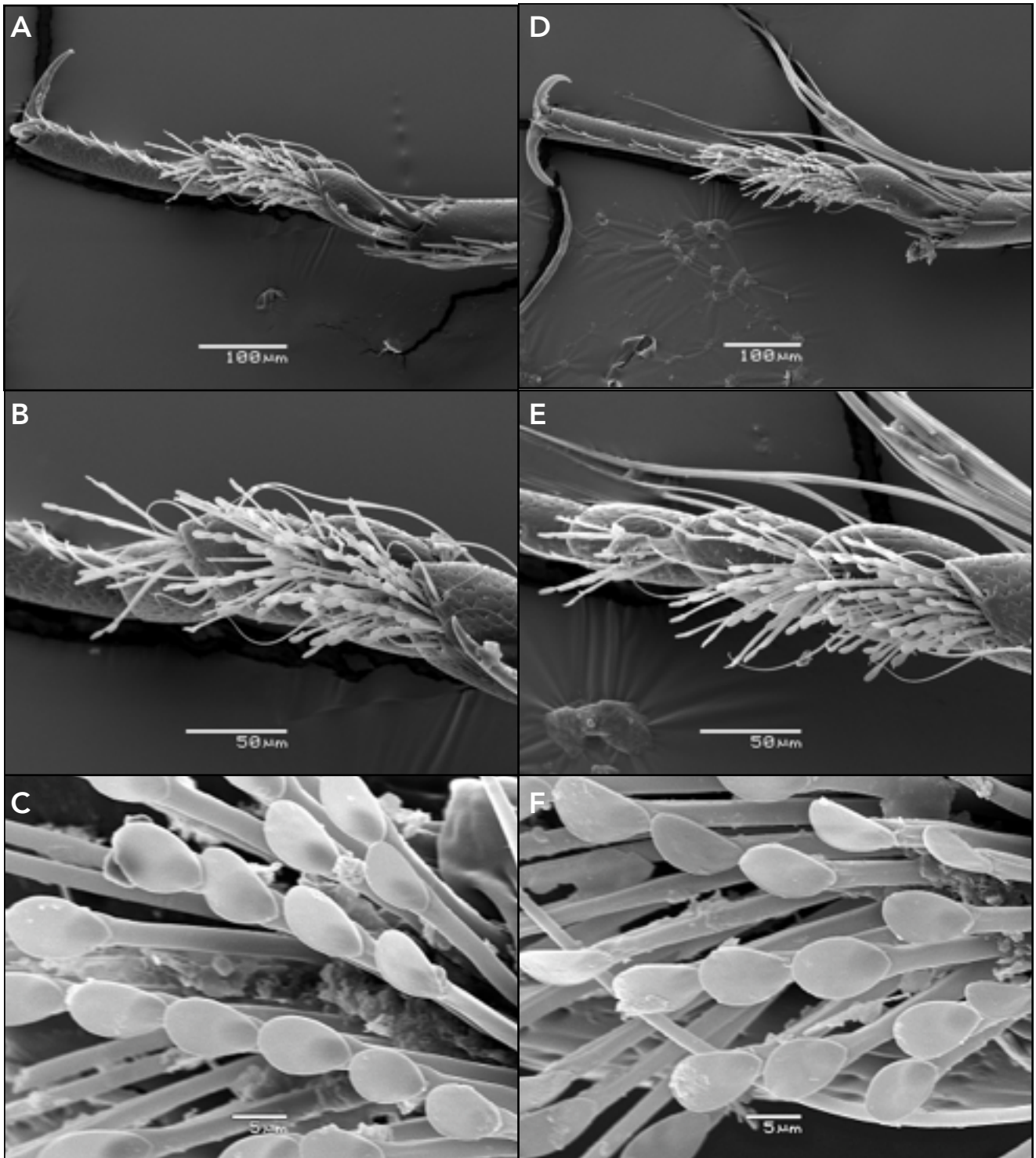
Females	Males
<i>H. africanus</i>	<i>H. africanus</i>
<i>H. immaculatus</i>	<i>H. immaculatus</i>
<i>H. lineatocollis</i>	<i>H. lineatocollis</i>
<i>H. mucronatus</i>	<i>H. ruficollis</i>
<i>H. obliquus</i>	
<i>H. ruficollis</i>	

## Results

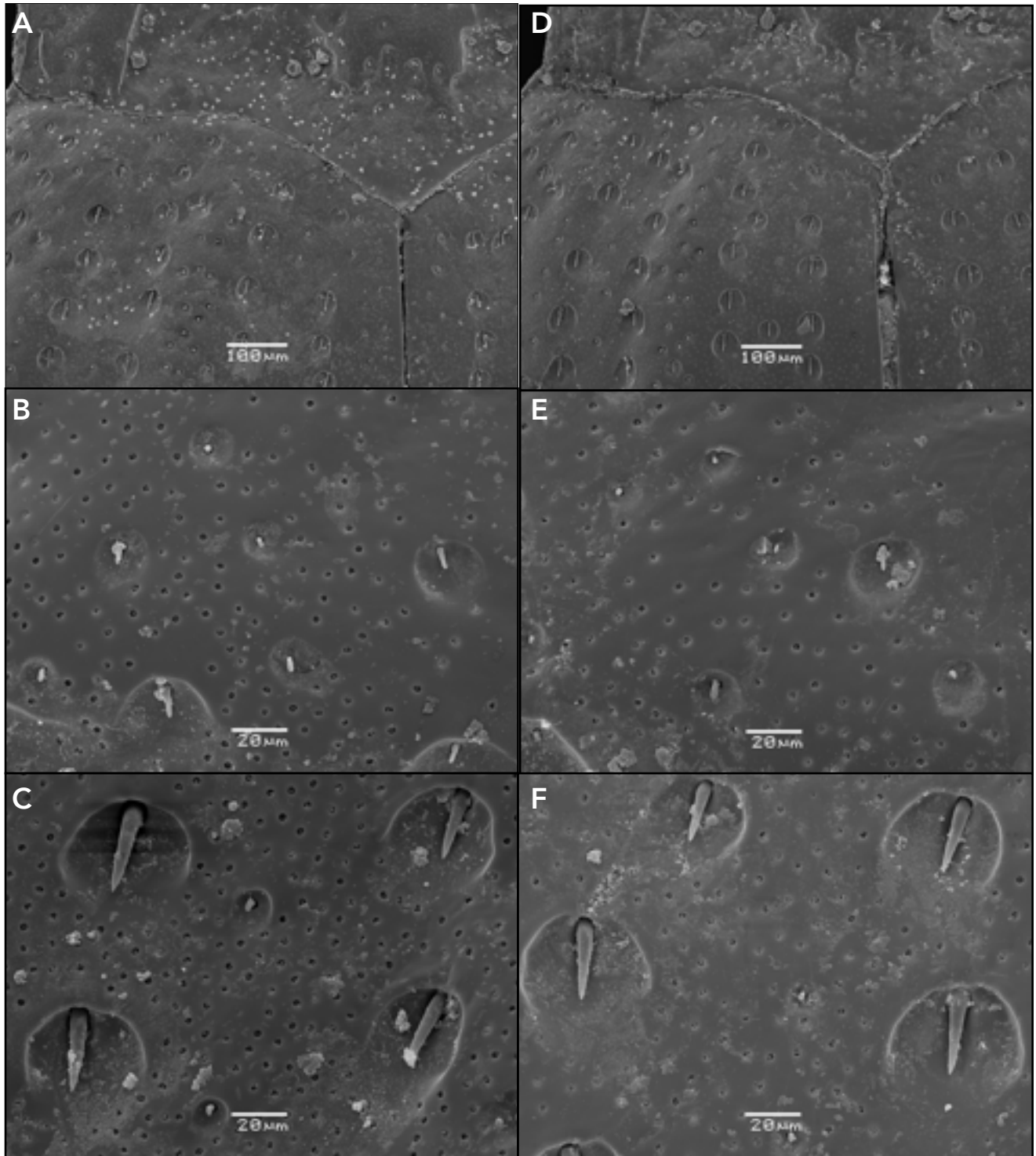
### Scanning electron microscopy images



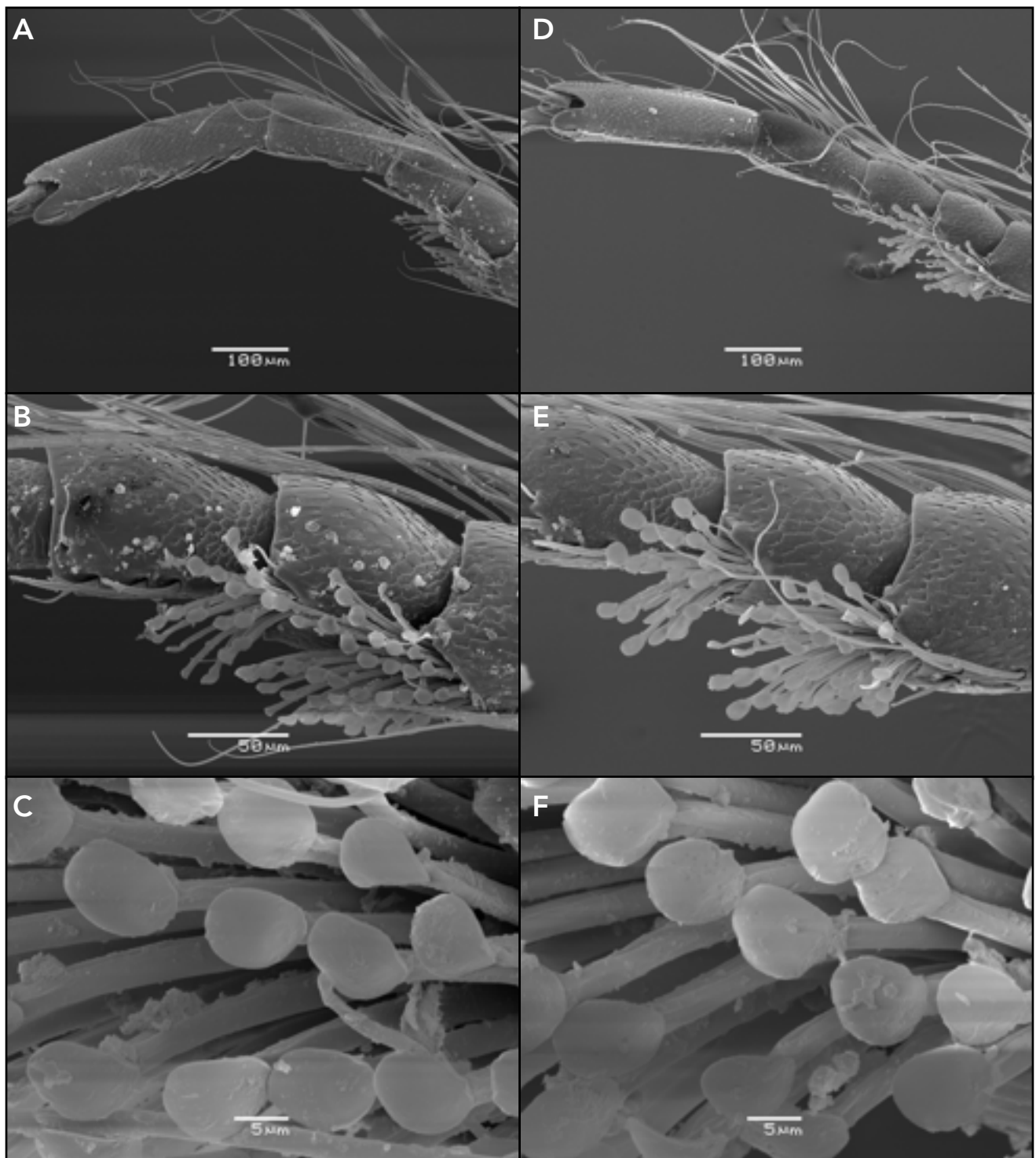
**Figure 2.** Dorsal surface of *Algophilus lathridioides*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.



**Figure 3.** *Algophilus lathridioides* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.

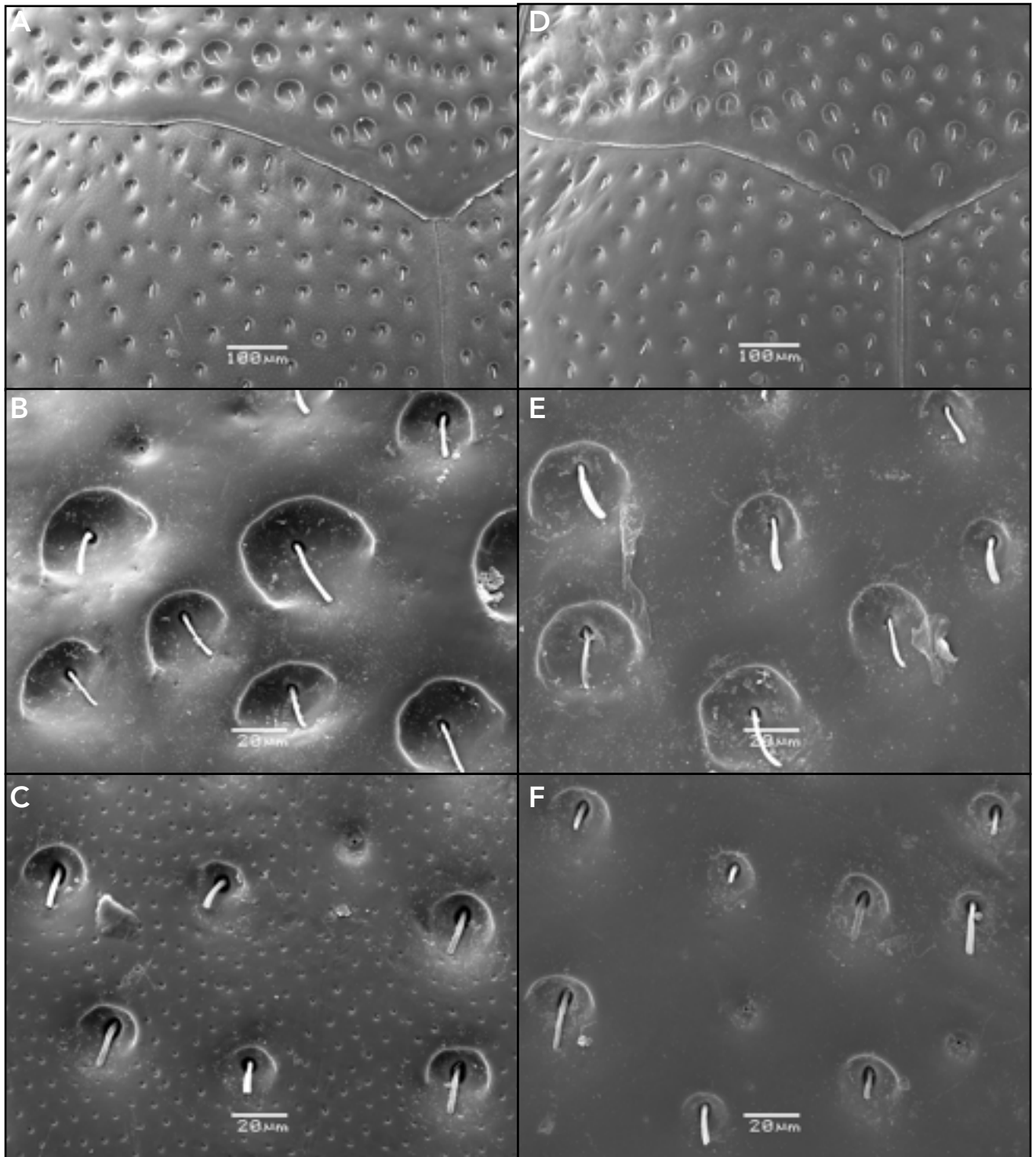


**Figure 4.** Dorsal surface of *Brychius elevatus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.

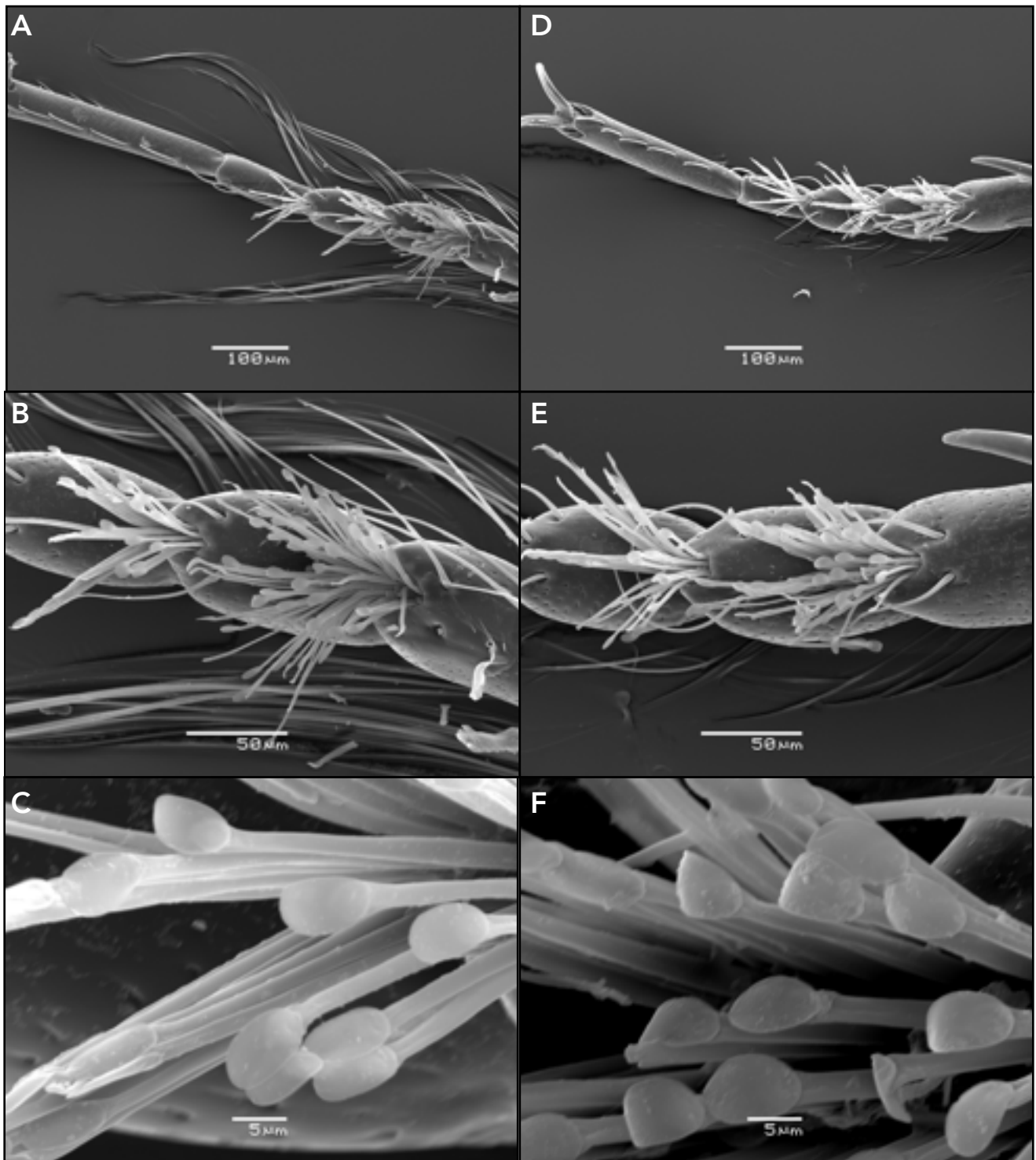


**Figure 5.** *Brychius elevatus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.



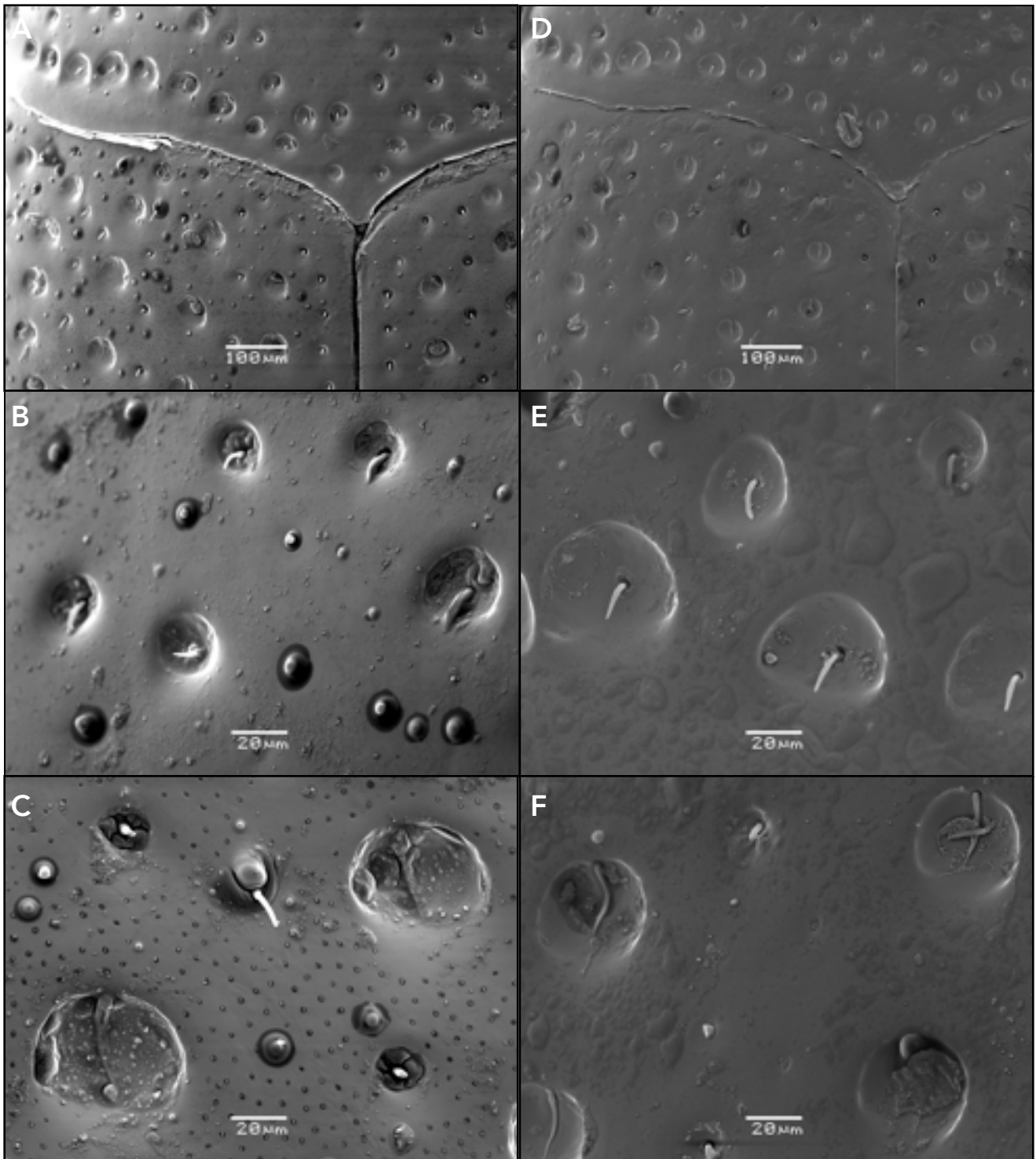


**Figure 6.** Dorsal surface of *Haliphus africanus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.

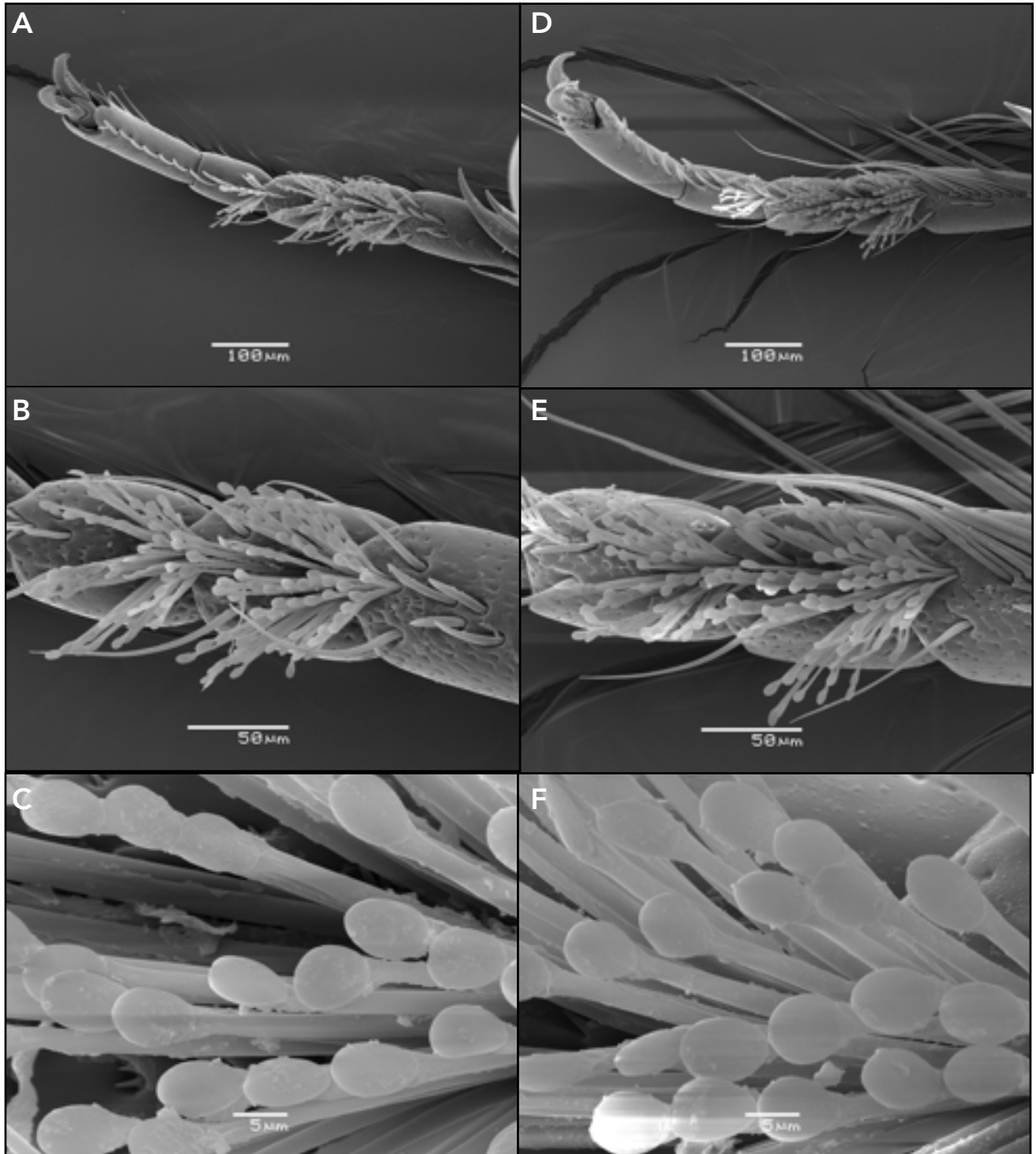


**Figure 7.** *Haliplus africanus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.

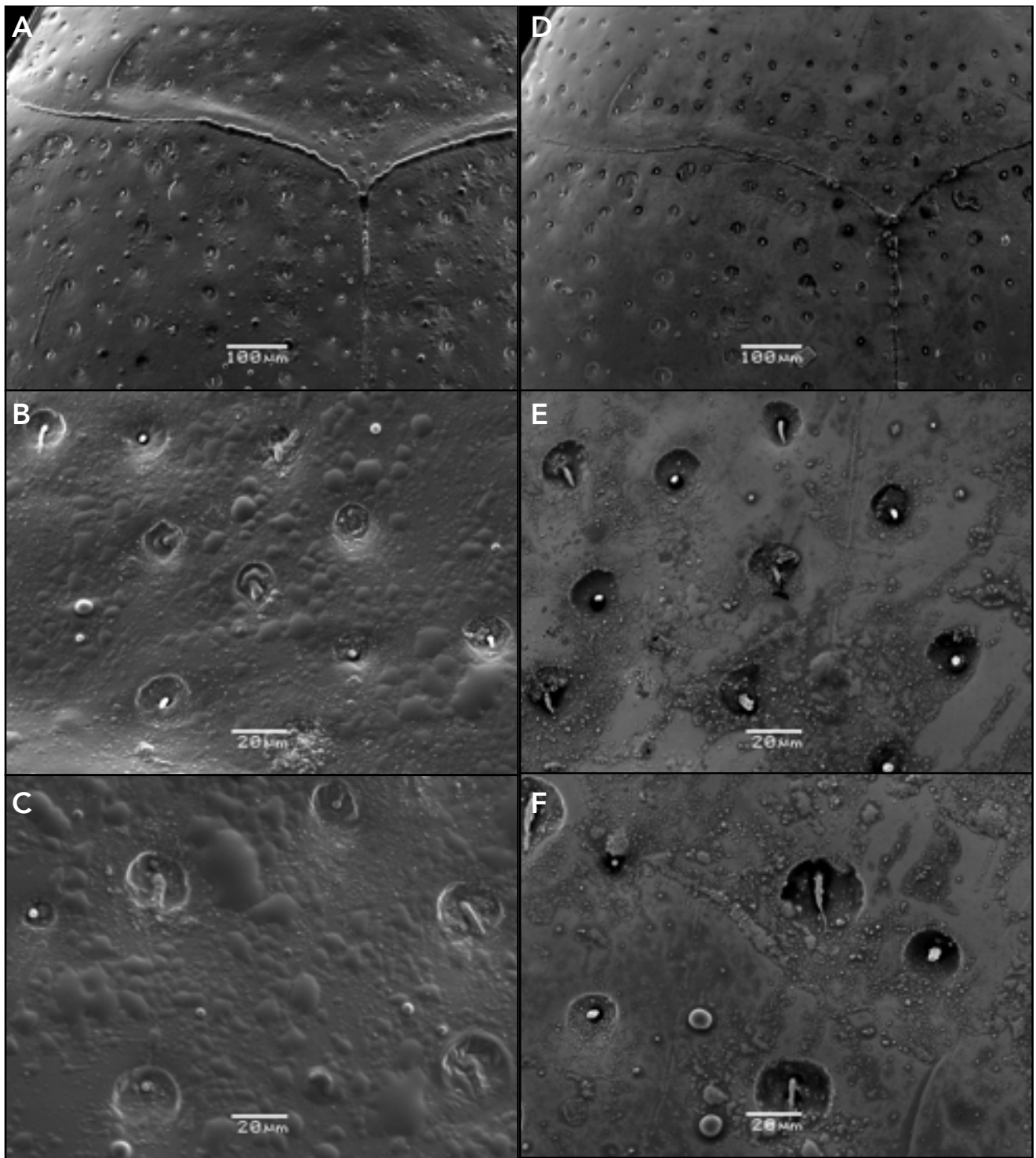




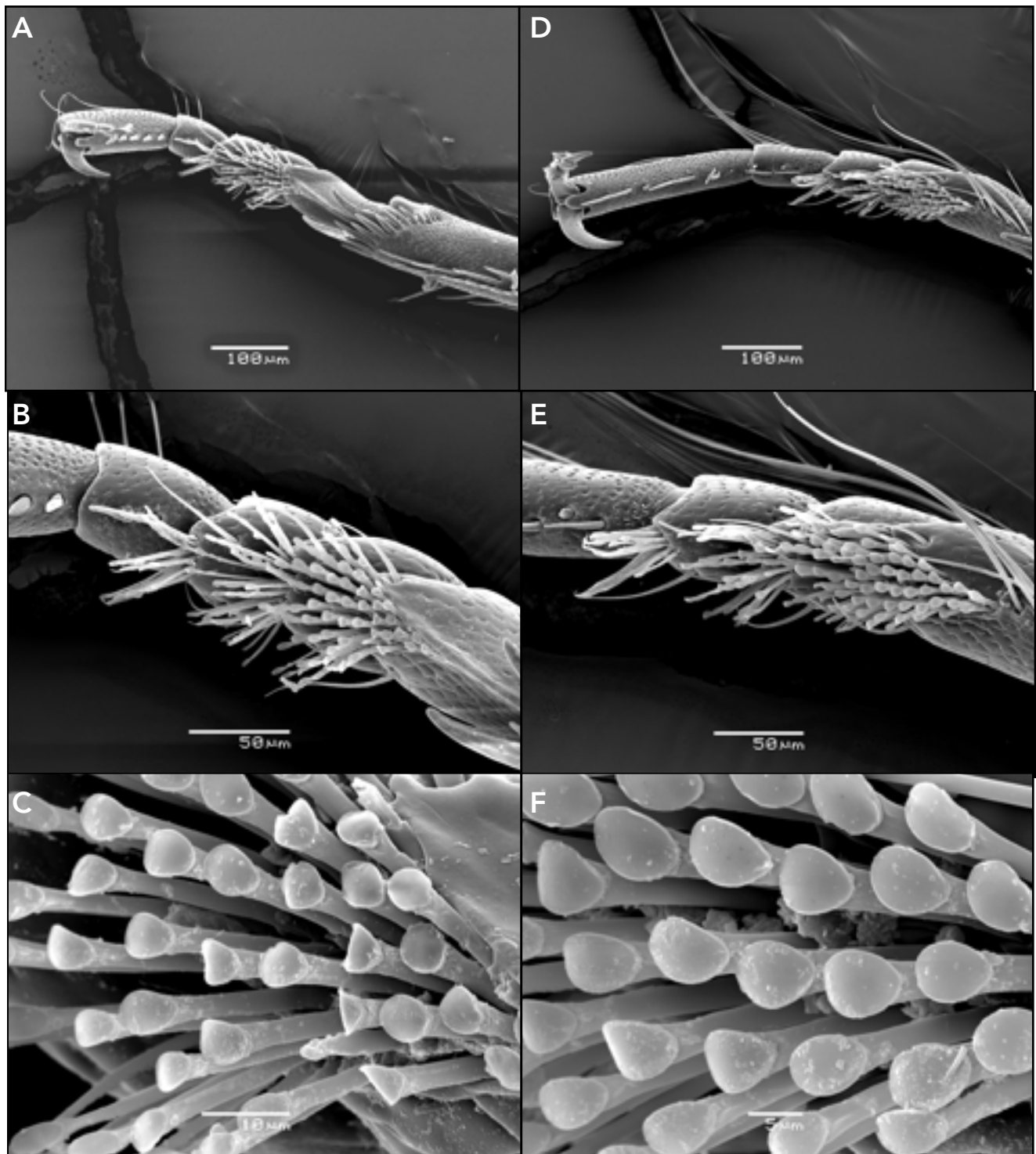
**Figure 8.** Dorsal surface of *Haliphus flavicollis*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.



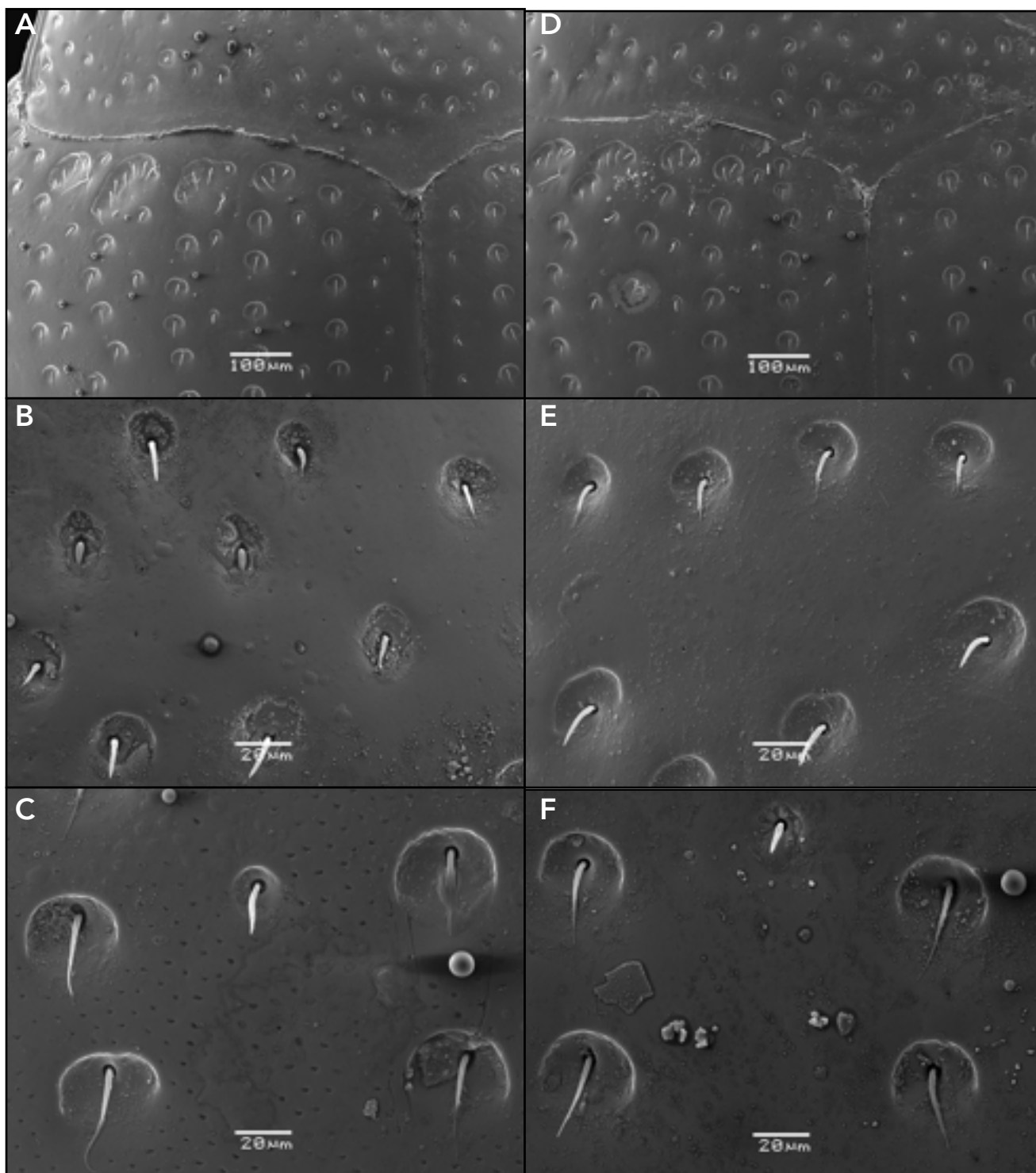
**Figure 9.** *Haliplus flavicollis* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.



**Figure 10.** Dorsal surface of *Haliphus immaculatus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.

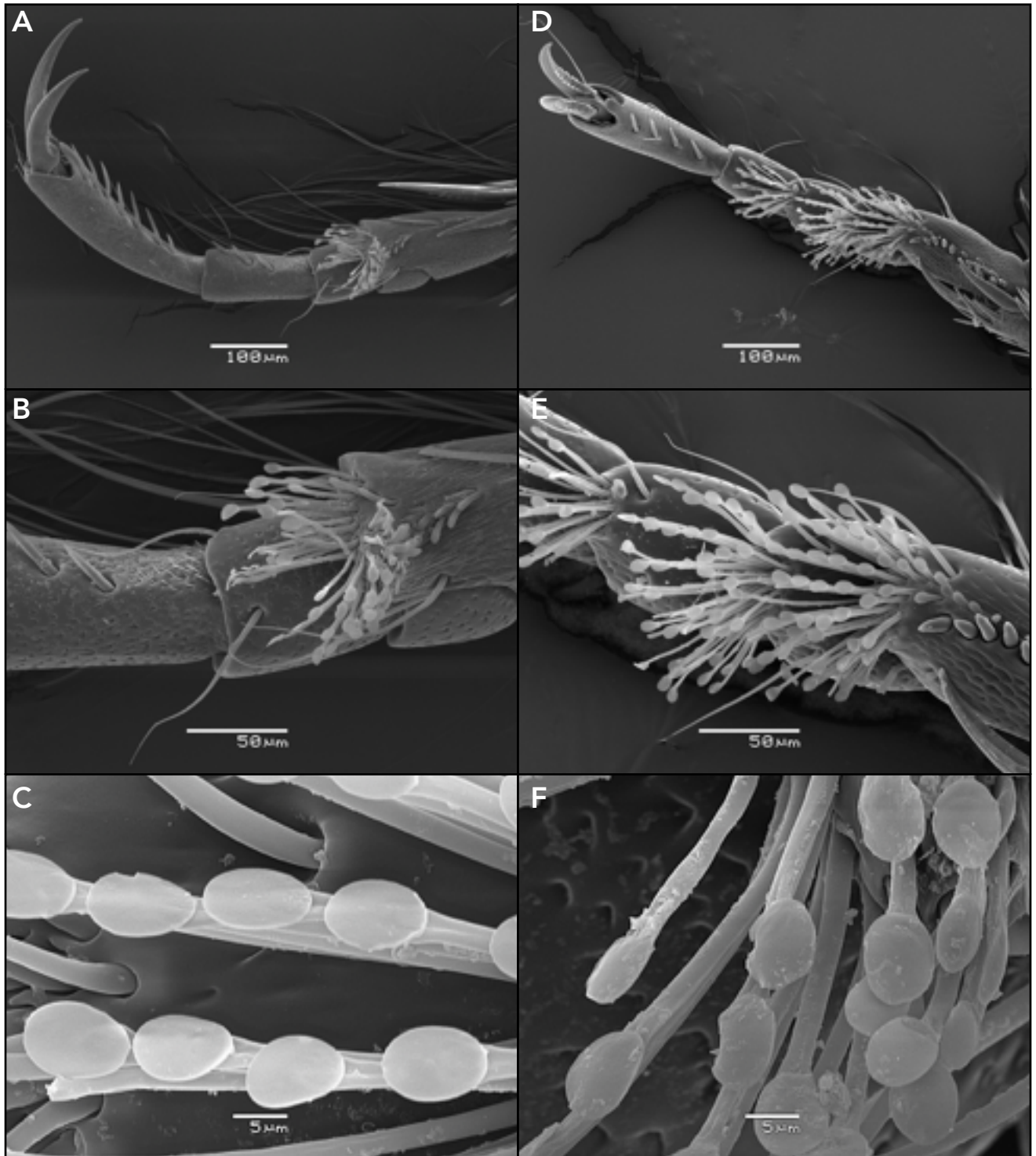


**Figure 11.** *Haliplus immaculatus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of suckers. Scale bars are given bottom middle of each micrograph.

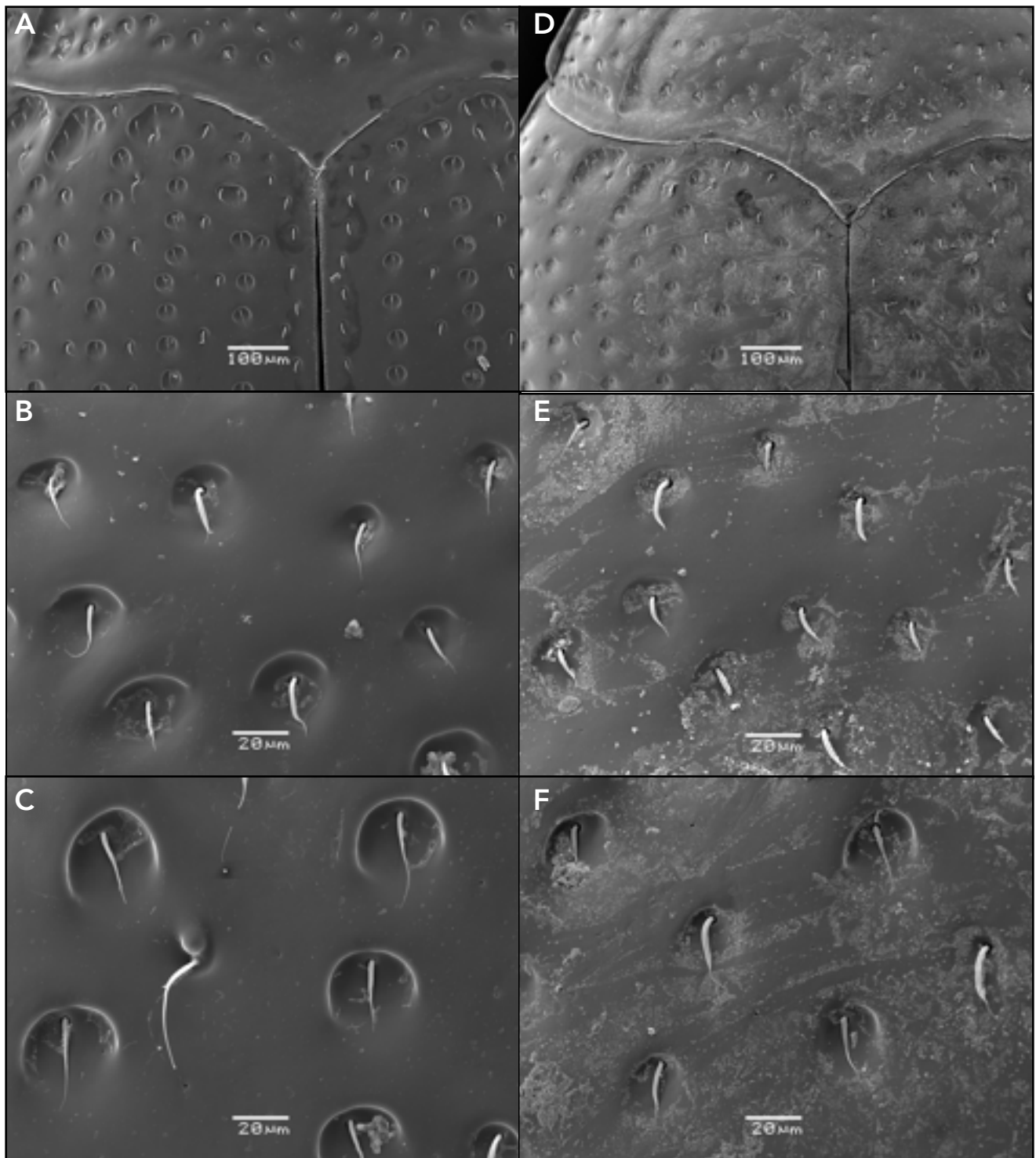


**Figure 12.** Dorsal surface of *Haliphus laminatus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.

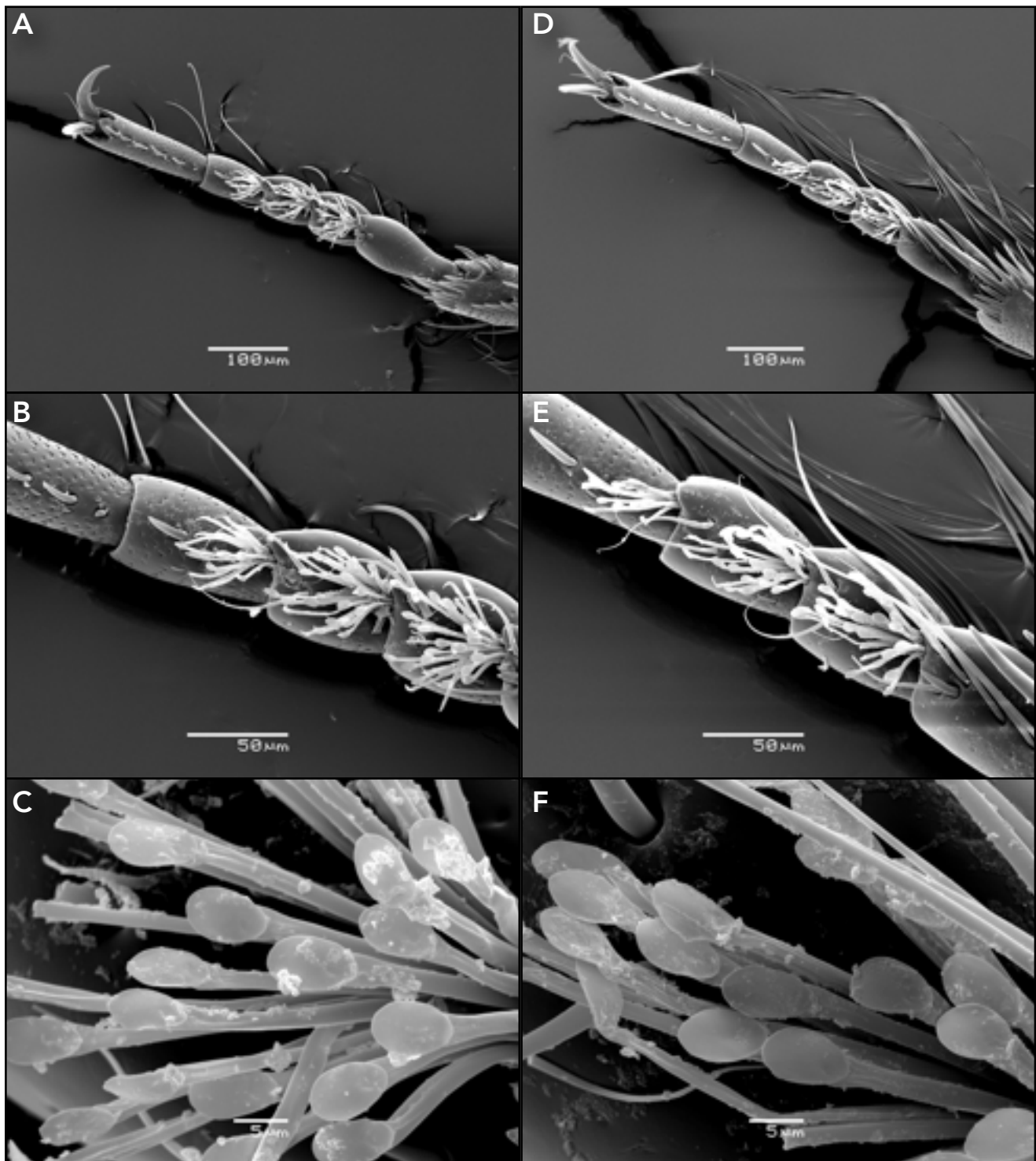




**Figure 13.** *Haliplus laminatus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.

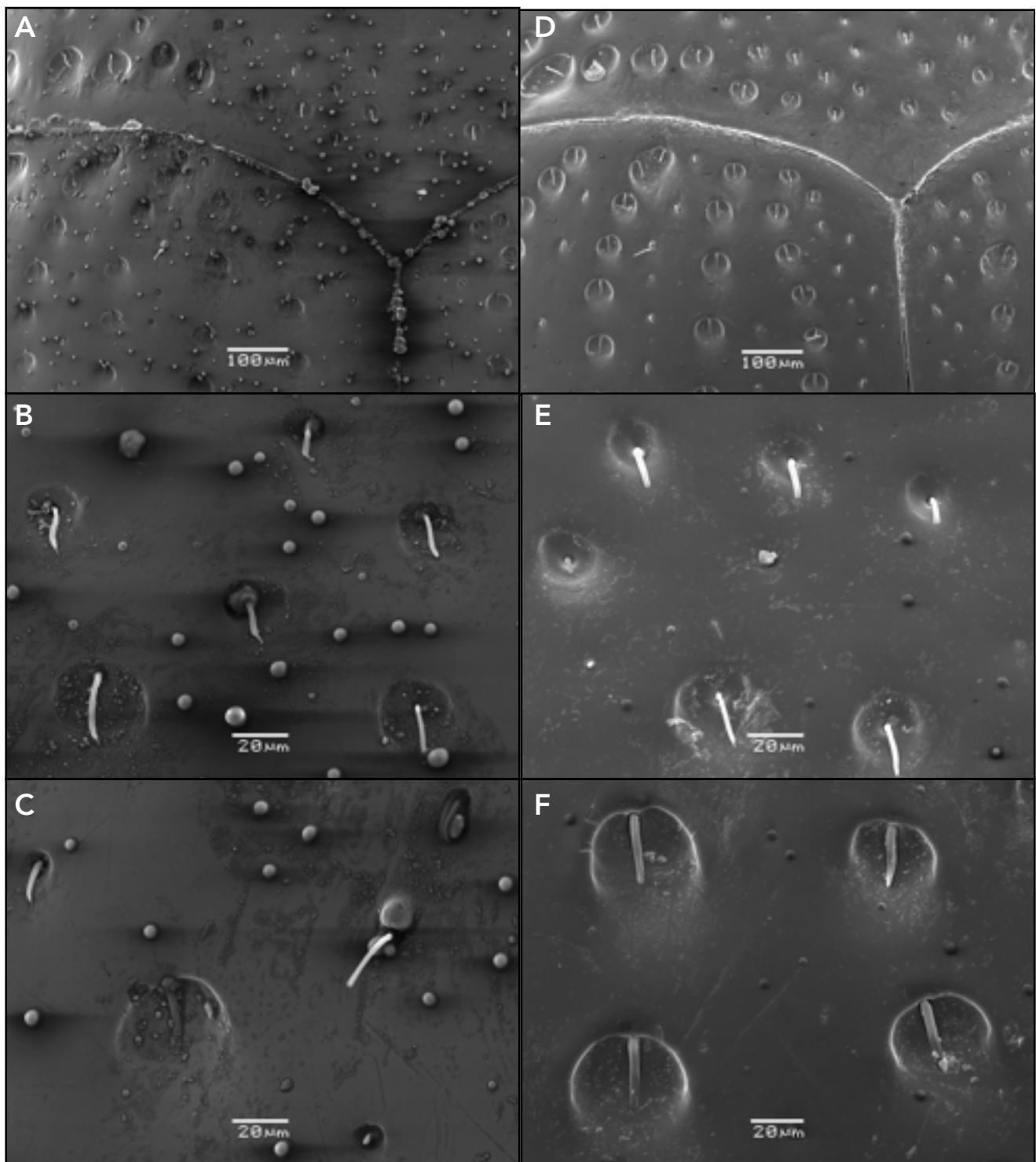


**Figure 14.** Dorsal surface of *Haliphus lineaticollis*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.

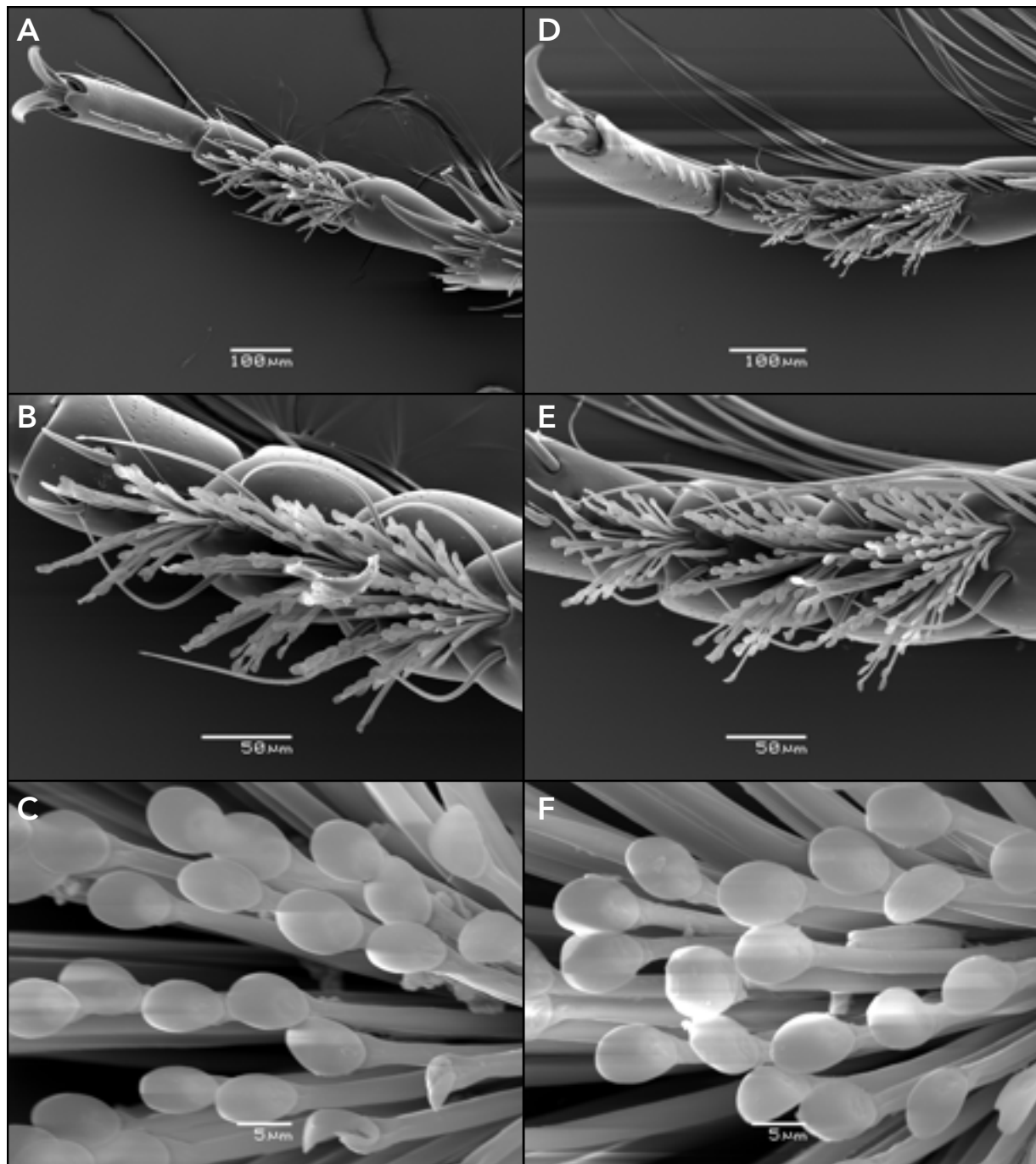


**Figure 15.** *Haliplus lineaticollis* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male suckers. Scale bars are given bottom middle of each micrograph.

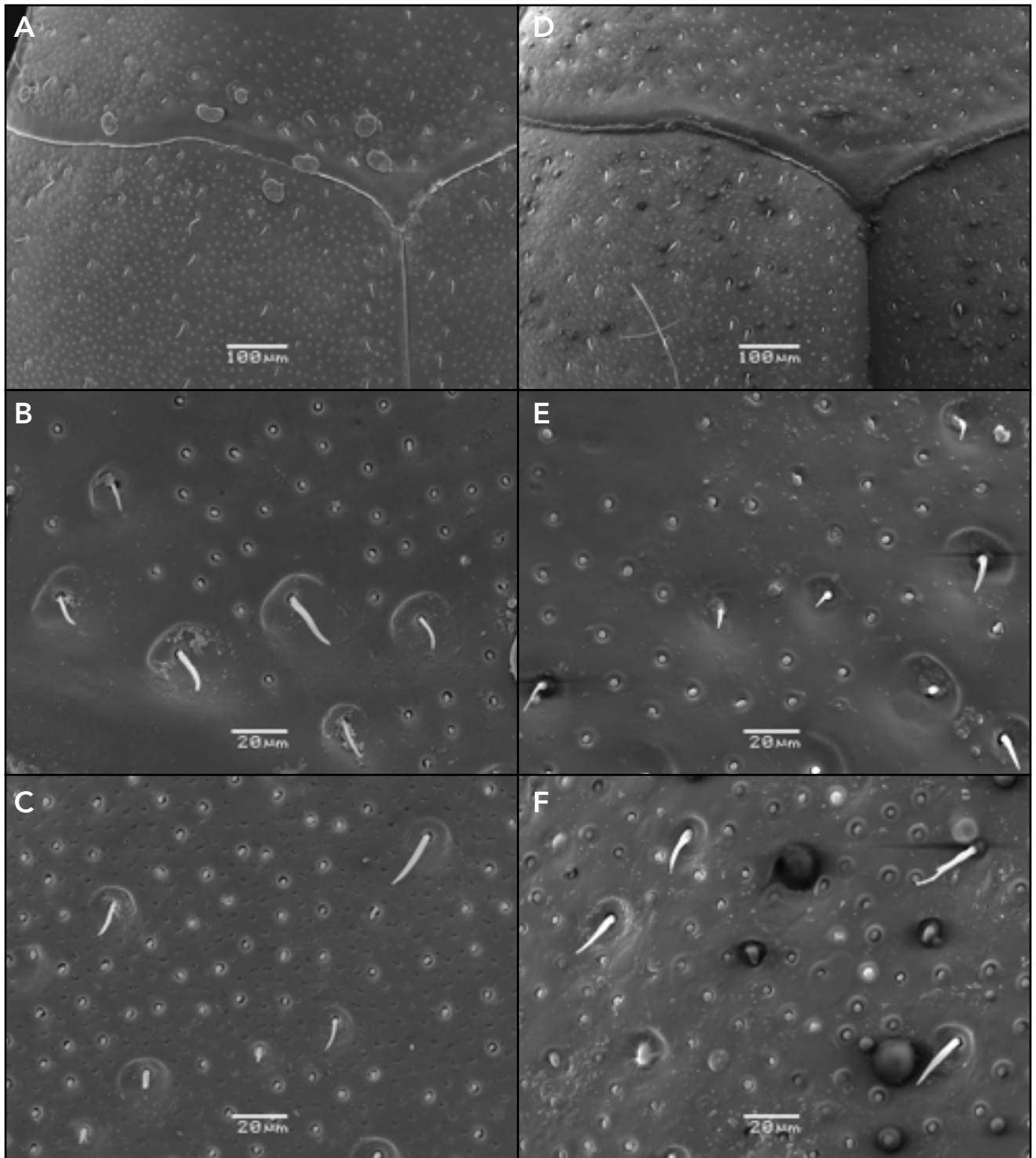




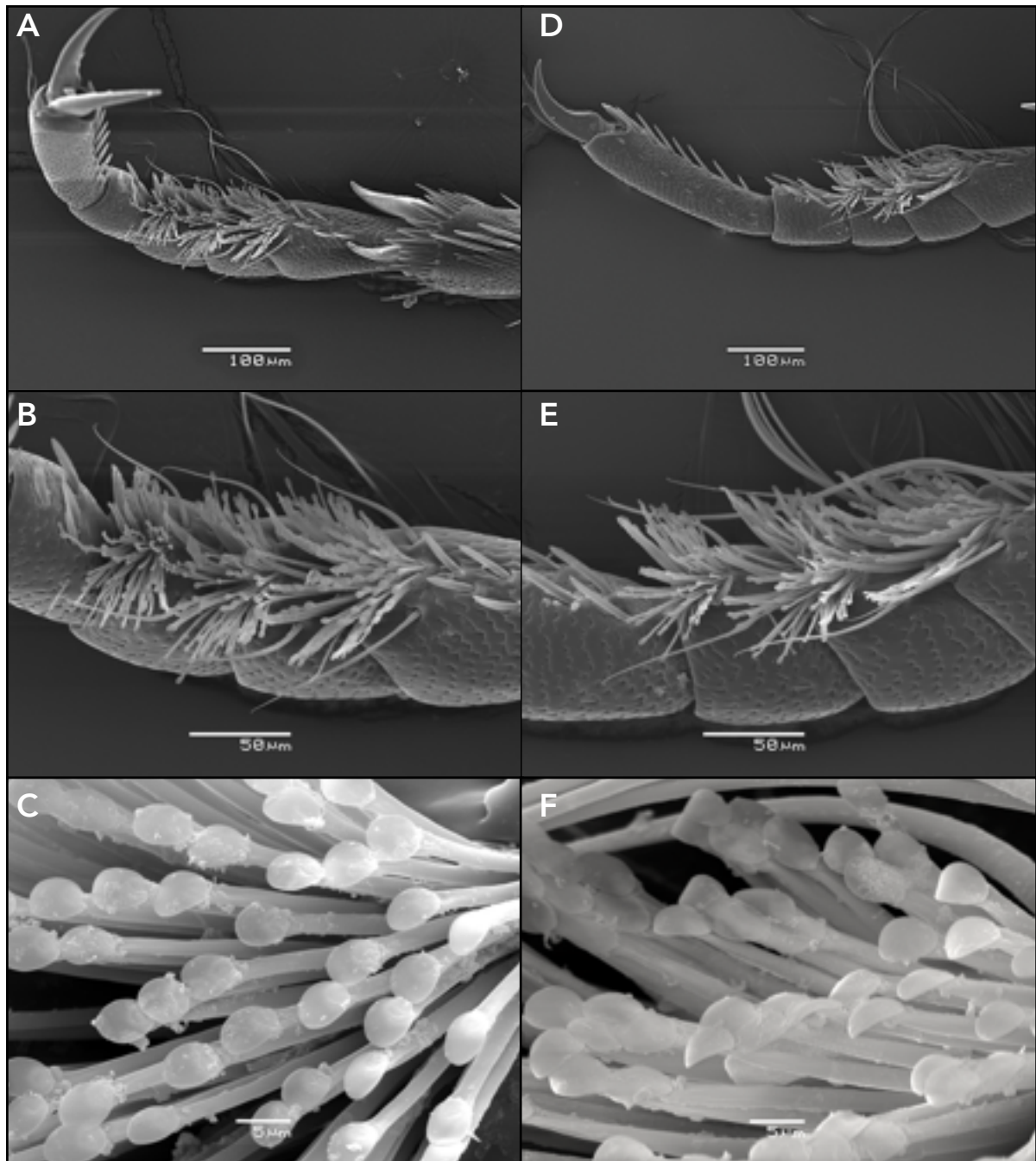
**Figure 16.** Dorsal surface of *Haliphus mucronatus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.



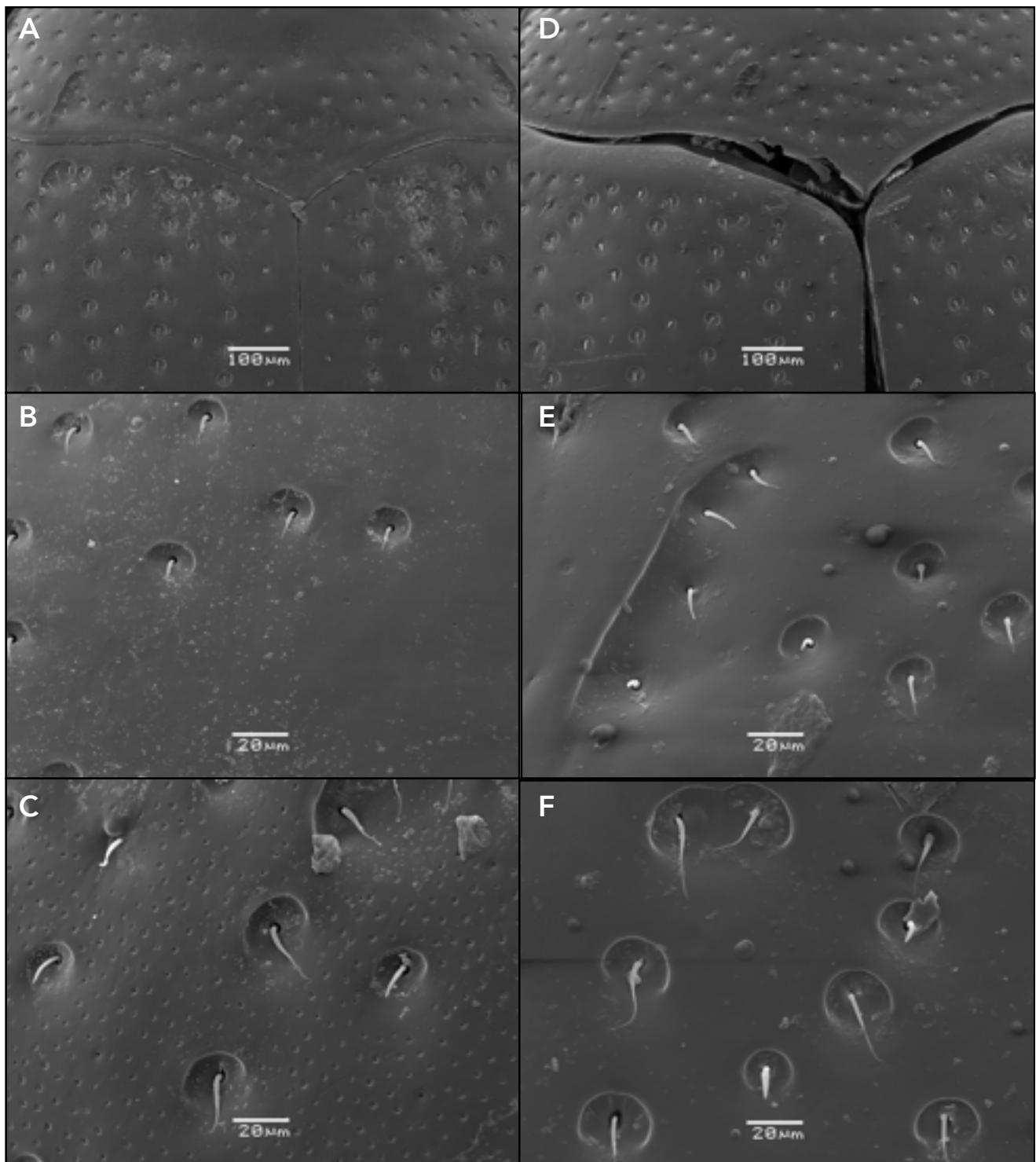
**Figure 17.** *Haliplus mucronatus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of foreleg suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of male mid-leg suckers. Scale bars are given bottom middle of each micrograph.



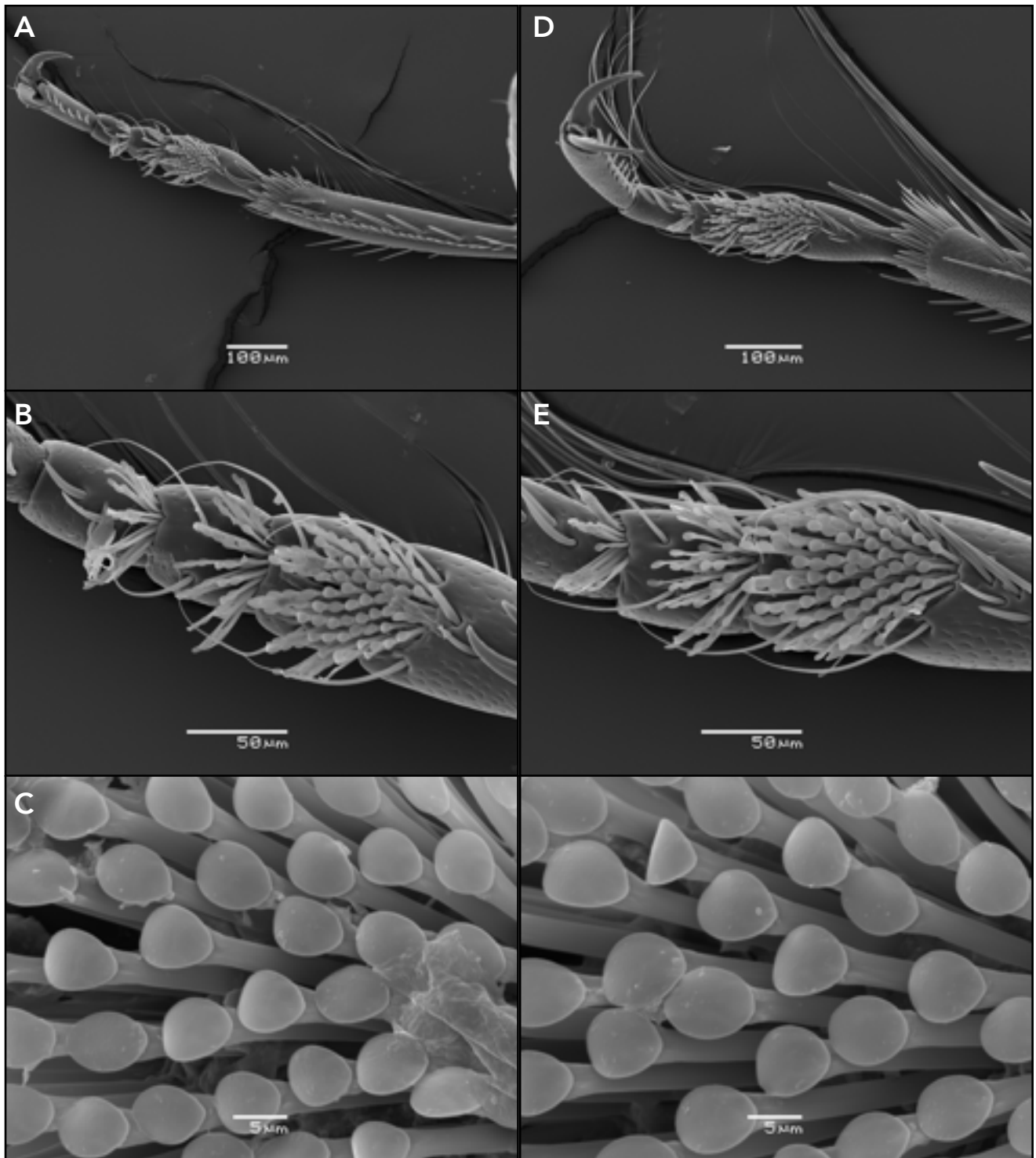
**Figure 18.** Dorsal surface of *Haliphus obliquus*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.



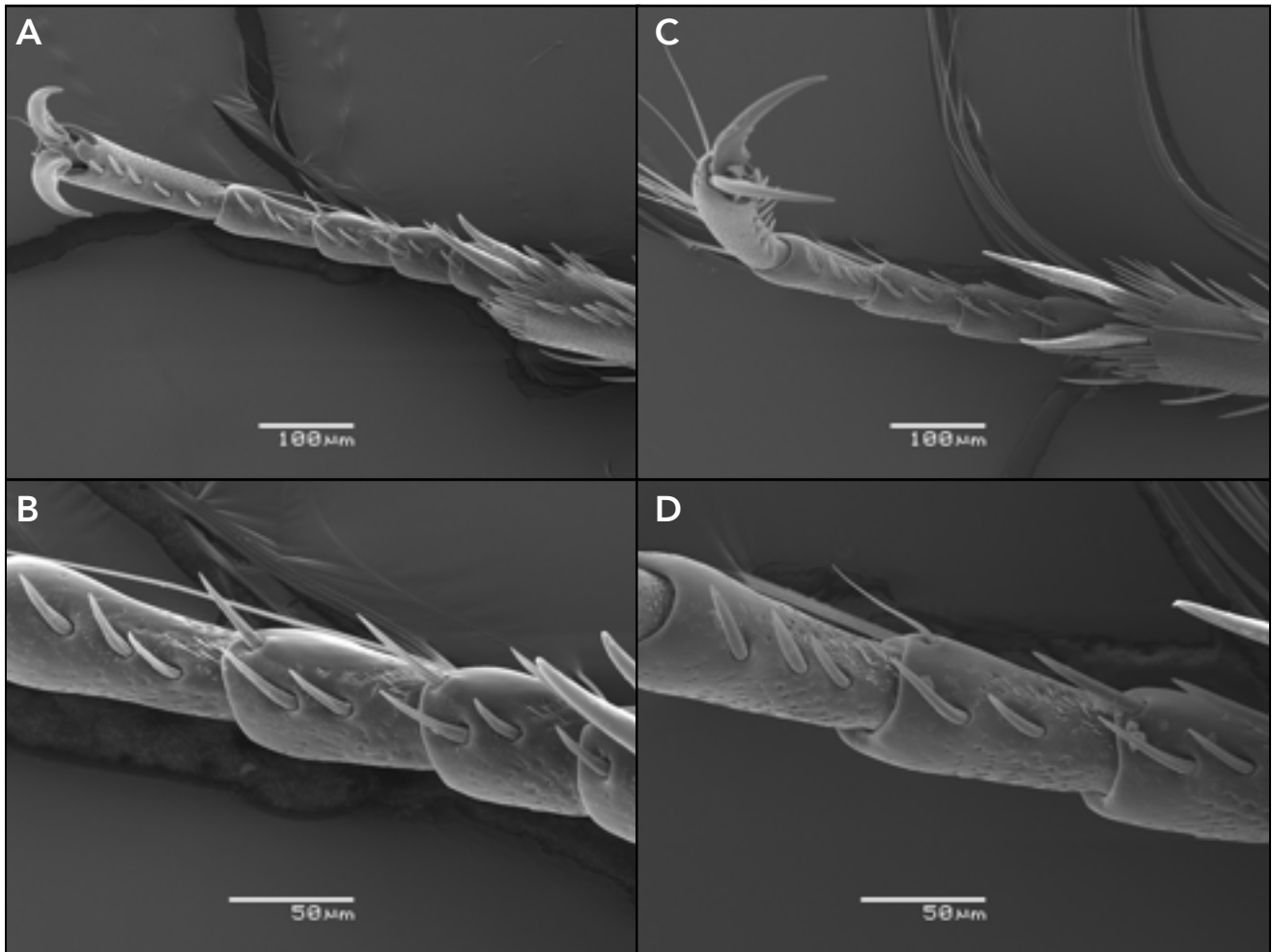
**Figure 19.** *Haliplus obliquus* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of foreleg suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of mid-leg suckers. Scale bars are given bottom middle of each micrograph.



**Figure 20.** Dorsal surface of *Haliplus ruficollis*. **A:** Female dorsal surface. **B:** Female pronotal disc. **C:** Female elytral disc. **D:** Male dorsal surface. **E:** Pronotal disc. **F:** Male elytral disc. Scale bars are given bottom middle of each micrograph.



**Figure 21.** *Haliplus ruficollis* male fore and mid-legs. **A:** Foreleg. **B:** Foreleg tarsal suckers. **C:** Close up of foreleg suckers. **D:** Mid-leg. **E:** Mid-leg suckers. **F:** Close up of mid-leg suckers. Scale bars are given bottom middle of each micrograph.



**Figure 22.** *Haliplus laminatus* female fore and mid-legs. **A:** Foreleg. **B:** Foreleg showing absent suckers. **C:** Mid-leg. **D:** Mid-leg showing absent suckers. Females were not studied quantitatively, this figure allows comparison to male secondary structures. Scale bars are given bottom middle of each micrograph.

### Micrograph observations

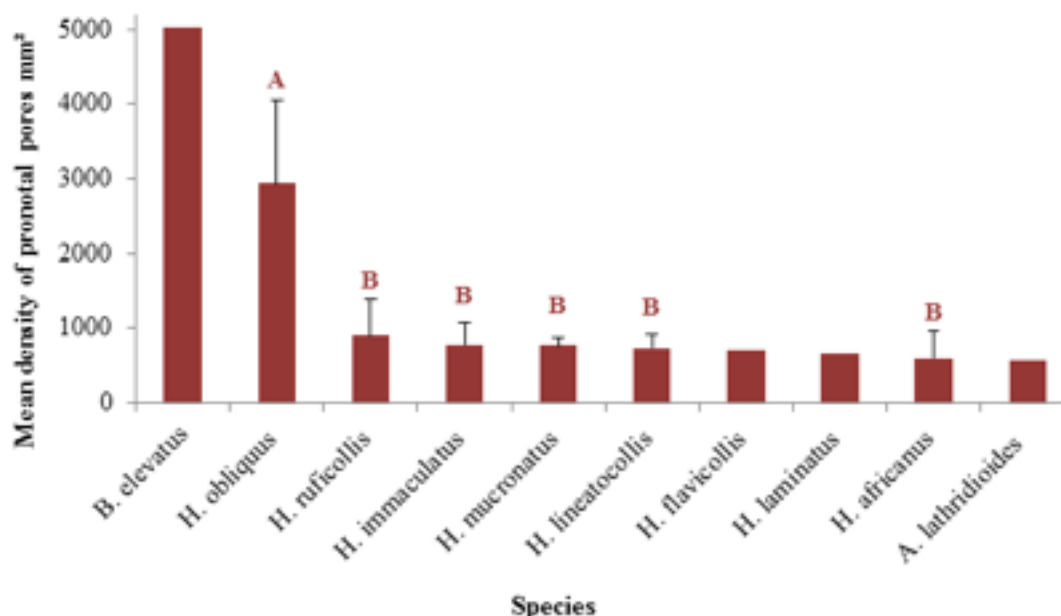
Observations of the SEM micrographs illustrate key differences in morphology between males and females. Some species have evident differentiation in pore density between males and females. In *H. africanus* (Fig. 6), *H. flavicollis* (Fig. 8), *H. immaculatus* (Fig. 10), *H. laminatus* (Fig. 12), *H. mucronatus* (Fig. 16) and *H. ruficollis* (Fig. 20) it was observed that females have higher density of pores than males. *H. lineaticollis* (Fig. 14) males and females do not appear to have large differences in pore densities. *B. elevatus* (Fig. 4) and *H. obliquus* (Fig. 18) males and females both have a relatively high density of pores.



## Statistical analysis

### Female dorsal surface

Species that were statistically analysed are represented on the histograms by the post hoc analysis (Table 2). The remaining species were graphed to allow comparison but were not statistically analysed. The results show that females differed between species significantly in the density of pores on the dorsal surface. Species also differed in the total density of pores on the pronotal disc (Fig. 23) (One-way ANOVA:  $F_{5,13} = 5.78$ ;  $P = 0.005$ ). Post-hoc analysis using Tukey's tests indicated that *H. obliquus* had significantly greater pore density than the other species examined. Fig. 23 indicates that *B. elevatus* has a high pore density, the remaining species do not differ significantly from each other.

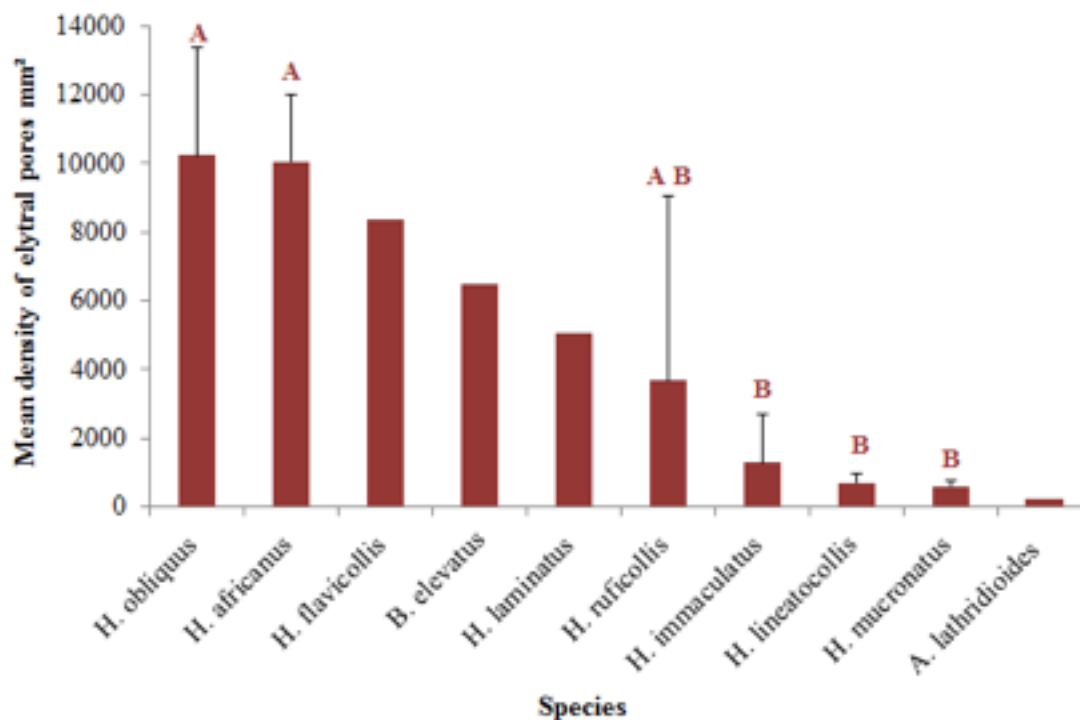


**Figure 23.** Mean pronotal pore density mm<sup>2</sup>. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

Analysis shows that females differed in total pore density on the elytra (One-way ANOVA:  $F_{5,13} = 6.95$ ;  $P = 0.002$ ) (Fig. 24). Post-hoc analysis using Tukey's tests showed that *H. obliquus* and *H. africanus* had the highest pore densities and were very similar to each other. *H. mucronatus* and *H. lineatocollis* had the lowest pore density and differed significantly from the rest of the species studied. Graphical observations suggest that *H. flavicollis* also had a

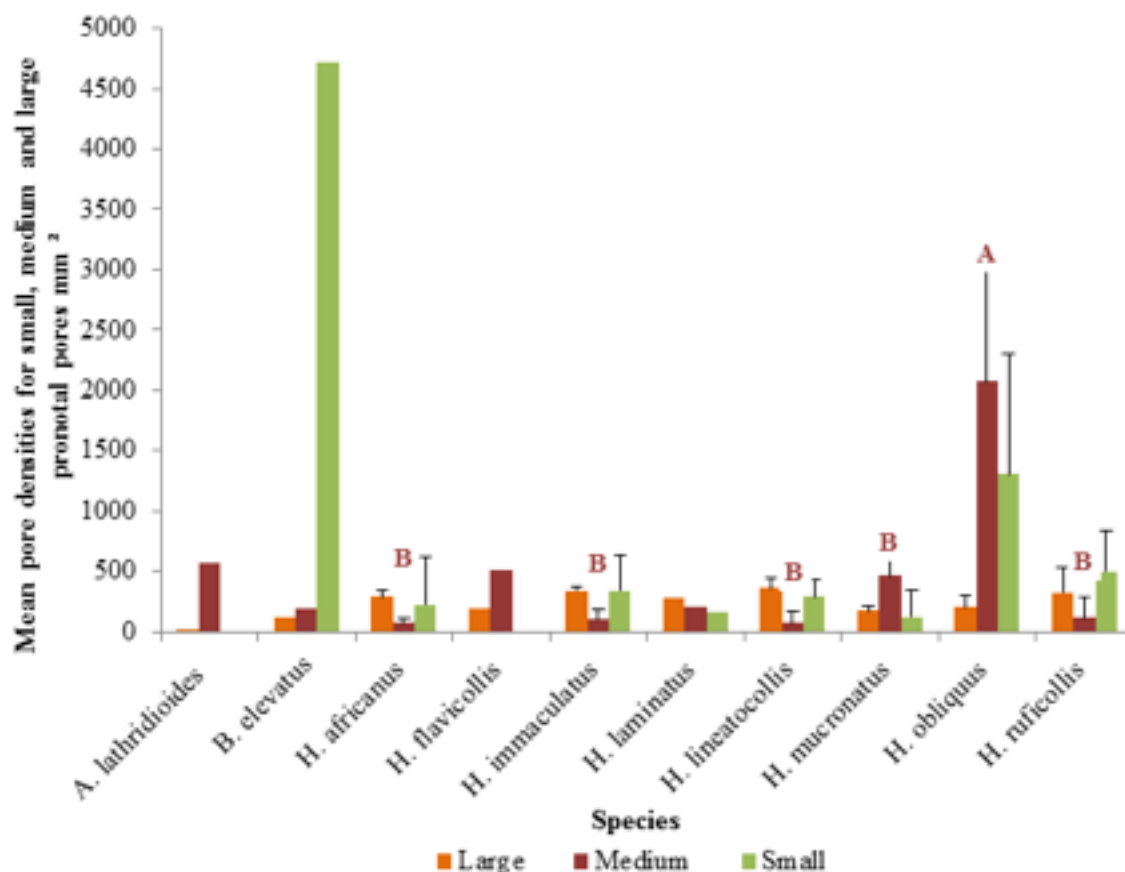


high density of elytral pores with a small deviation bar. The large standard deviation bars represented for *H. ruficollis* indicate that there is large variability, this could be due to the nature of biological data.



**Figure 24.** Mean elytral pore density mm<sup>2</sup>. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

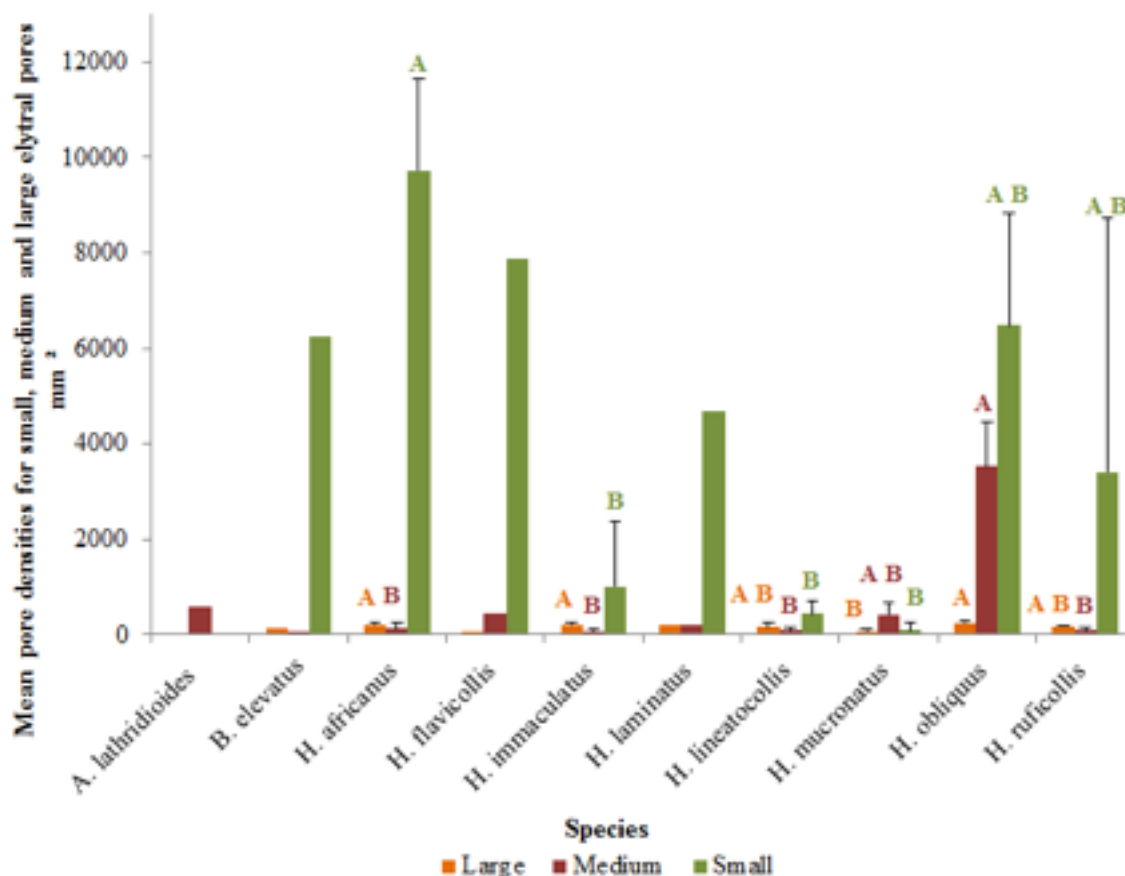
There were three different sized pores identified during the analysis; small, medium and large. Species did not differ in the density of large pronotal pores (One-way ANOVA:  $F_{5,13} = 1.88$ ;  $P = 0.166$ ) (Fig. 25). There was a significant difference reported for medium sized pores between species. Post hoc analysis indicated that *H. obliquus* had the highest density of medium sized pores (One-way ANOVA:  $F_{5,13} = 11.78$ ;  $P < 0.001$ ). There was no significant difference found in the density of small pores on the pronotum between females (One-way ANOVA:  $F_{5,13} = 2.47$ ;  $P = 0.088$ ) (Fig. 25). However, observation of the histogram suggests *H. obliquus* had a high small pore density, this was similarly evident for *B. elevatus* (this species was not statistically analysed), this species has the greatest small pore density than the other species small pores densities on the elytral disc. Statistical analysis of this species in the future would be beneficial to this field of study.



**Figure 25.** Mean pore density for small, medium and large pores on the pronotal disc. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

Statistical analyses reported a significant difference in the densities of large pores on the elytral disc, *H. obliquus*, *H. immaculatus* and *H. africanus* had the highest densities of large pores and were similar to each other in those densities (One-way ANOVA:  $F_{5,13} = 4.34$ ;  $P = 0.015$ ) (Fig. 26). The density of medium sized pores on the elytra reported a highly significant difference between species (One-way ANOVA:  $F_{5,13} = 9.99$ ;  $P < 0.001$ ). Post hoc analysis indicated that *H. obliquus* again had the highest density and *H. lineatocollis* had significantly fewer than the other species examined. The remaining species (including the species that were not statistically analysed) all had very similar medium pore densities. Species differed in the densities of small pores on the elytra as small pores were found to be more numerous than the other sized pores (One-way ANOVA:  $F_{5,13} = 5.44$ ;  $P = 0.006$ ). Post hoc analysis reports that *H. africanus* and *H. obliquus* differed from the rest of the species with the highest density. Observations of the histogram also indicate that *H. flavicollis* also has a high density of small

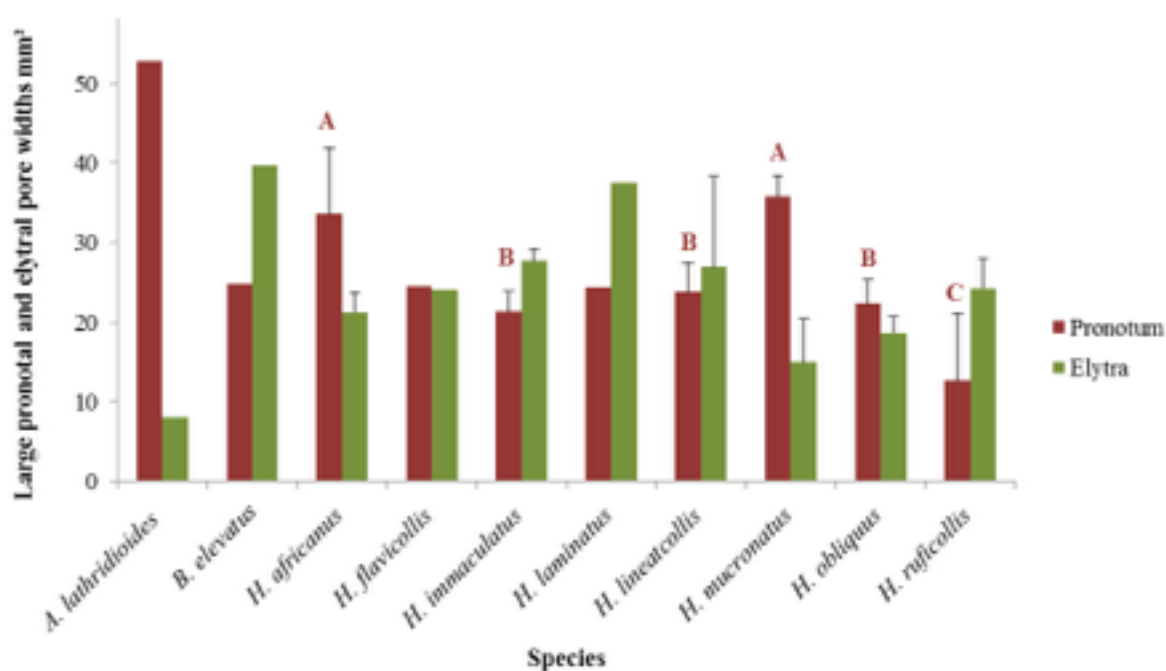
pores. Fig. 26 illustrates the vast increase in the densities of small pores on the elytra disc across species. Small pore densities are significantly higher in comparison with the pronotal disc. However, medium and large pore densities are higher on the pronotal disc. Observations of the micrographs suggest that there are differences in pore densities between sexes, particularly in the density of small sized pores on the elytra (Fig. 6, 8, 12 and 20). Small pores are absent in almost all males, with the exception of *B. elevatus* and *H. immaculatus*.



**Figure 26.** Mean pore density for small, medium and large pores on the elytral disc. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

Female large pore widths (Fig. 27) were found to differ significantly on the pronotal disc (One-way ANOVA:  $F_{5,70} = 20.51$ ;  $P < 0.001$ ). Post-hoc analysis using Tukey's tests indicated that *H. mucronatus* and *H. africanus* had the largest width of large pores, followed by *H. obliquus* and *H. lineatocollis* respectively. Observations of the histogram indicate that *A. lathridioides* had the largest size pores out of all the species. There was differentiation

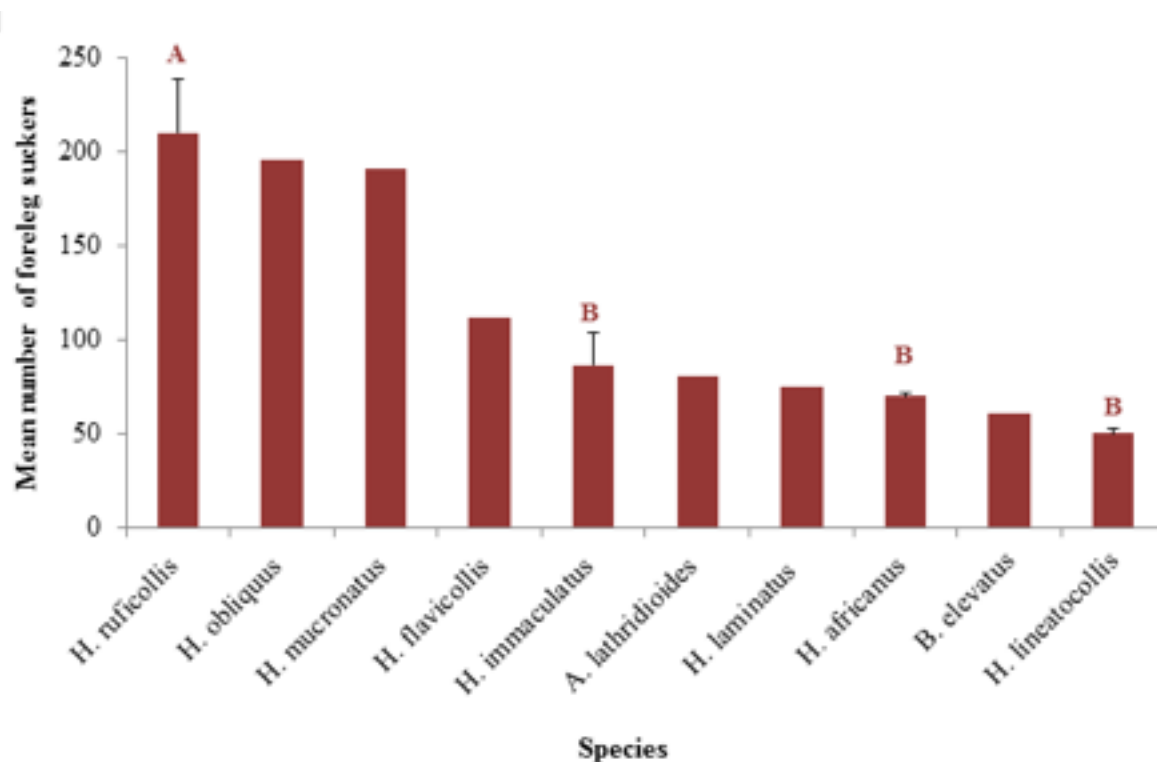
between species in the width of large pores on the elytral disc with a significant (Kruskal-Wallis:  $H=25.93$ ,  $df=5$ ,  $P<0.001$ ). *H. immaculatus* and *H. lineatocollis* had the most significantly large pores on the elytra. *H. laminatus* and *B.elevatus* followed with the second and third largest pores, further investigation of these species would be required to statistically confirm this. The remaining species did not differ significantly in pore size.



**Figure 27.** Female large pore width across species on the pronotum and elytra µm. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

#### Male secondary characters

Males differed between species significantly in the total number of foreleg suckers between males (One- way ANOVA:  $F_{3,11} = 66.26$ ;  $P < 0.001$ ) (Fig. 28). Post-hoc analysis indicated that *H. ruficollis* had a significantly greater number of foreleg suckers with *H. lineatocollis* having significantly fewer. The remaining species that were analysed did not differ significantly from one another. Observations of the histogram suggest that *H. obliquus* and *H. mucronatus* have numerous foreleg suckers. Fig. 28 illustrates that *H. obliquus* and *H. mucronatus* also had numerous foreleg suckers.

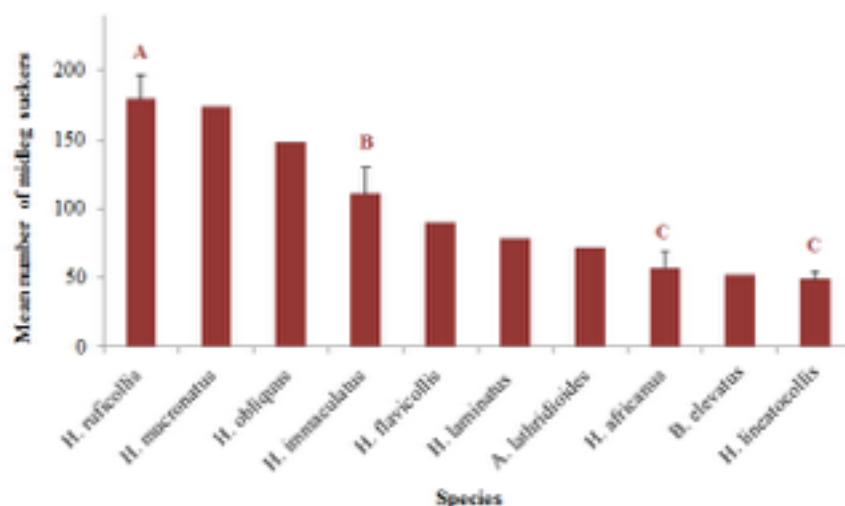


**Figure 28.** Mean number of foreleg suckers in males. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

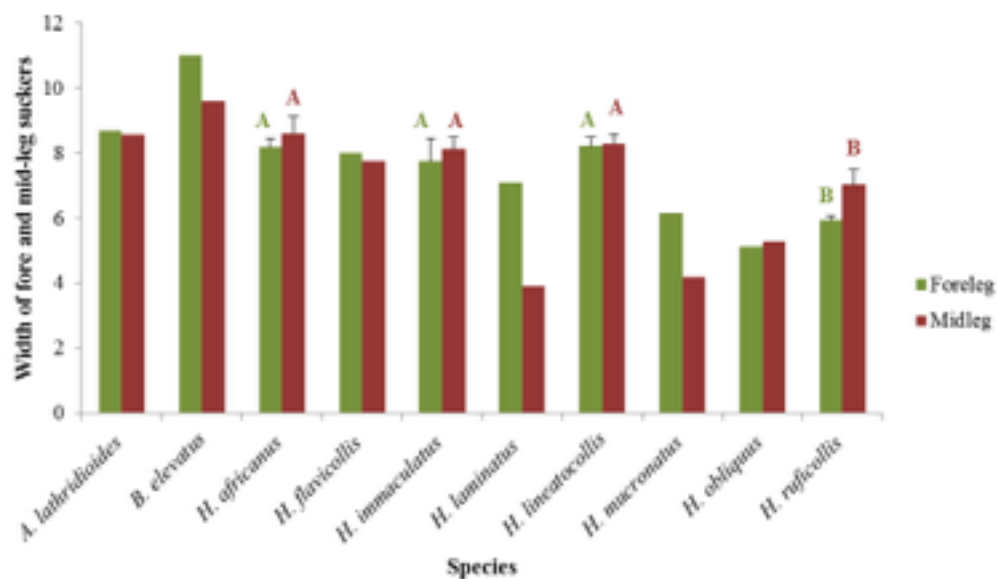
Males differed significantly between species in the total number of mid-leg suckers ( $F_{3,11} = 63.40$ ;  $P < 0.001$ ) (Fig. 29). Post-hoc analysis indicated that *H. ruficollis* had the highest number of mid leg suckers, followed by *H. immaculatus*, the remaining species did not differ significantly from one another. Examination of the histogram infers that *H. mucronatus* has numerous mid-leg suckers.

Male tarsal widths (Fig. 30) differed significantly between species ( $F_{3,71} = 68.95$ ;  $P < 0.001$ ). Post-hoc analysis inferred that *H. africanus*, *H. immaculatus* and *H. lineaticollis* did not differ significantly from each other, these species had the largest sucker width in comparison to the rest of the species. The width of mid-leg suckers reported a highly significant difference between species; post-hoc analysis using Tukey's test similarly indicated that *H. africanus*, *H. immaculatus* and *H. lineaticollis* had the largest sucker widths. Observation of Fig. 30 indicates that *B. elevatus* has the largest sucker width in comparison to the other species studied, statistical analysis would clarify this inference and *H. obliquus* has similarly smaller

sucker widths on both the fore and mid-leg.

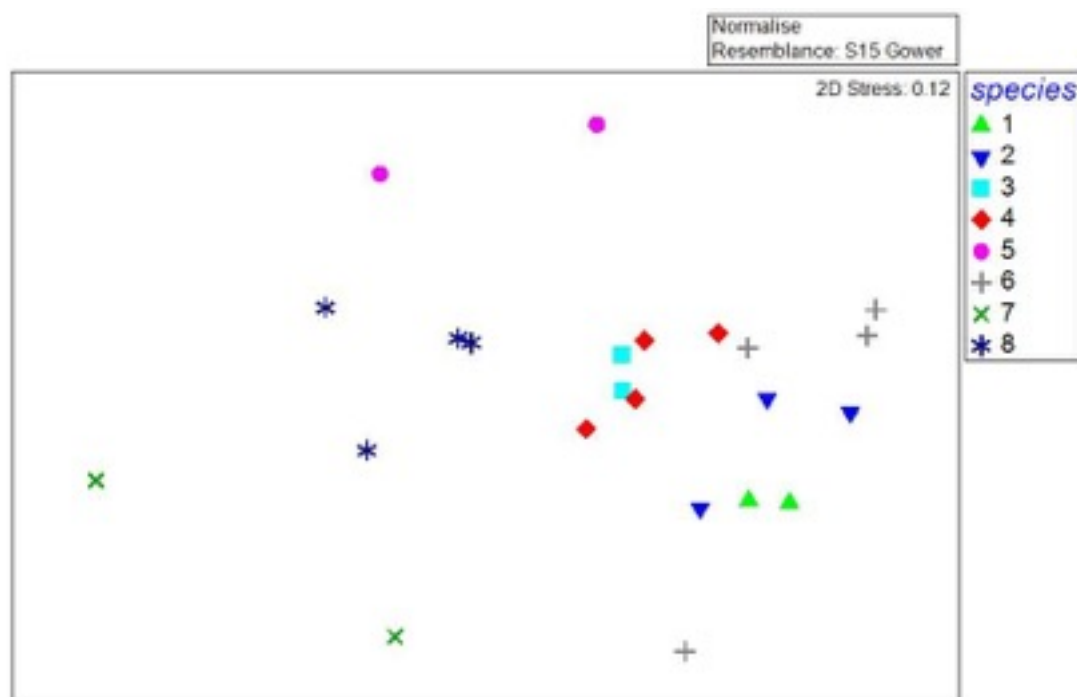


**Figure 29.** Mean number of mid-leg suckers in males. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.



**Figure 30.** Mean width of fore and mid-leg suckers across male species. Post-hoc analysis was applied to all histograms (represented by letters) where there was a significant difference between species. Means that do not share a letter are significantly different. Histograms without letters were not statistically analysed. Histograms represent the mean and standard deviation bars.

When analysing overall characters in males there was a highly significant difference reported between species (PERMANOVA:  $F_{7,15} = 5.6054$ ;  $P = 0.0001$ ). Fig. 31 illustrates the male species that differ in morphology, *H. lineaticollis* and *H. obliquus* differ significantly in morphology, followed by *H. ruficollis* which has some clustering amongst two of the individuals with the remaining two further apart. This analyses reported that female species did not differ from one another in overall morphological traits.



**Figure 31.** Non metric multidimensional scaling diagram (MDS) for the overall morphological similarities between males. The MDS diagram illustrates individual males of different species that are morphologically different, plots further away are more significantly different. Species: 1: *H. africanus*, 2: *H. flavicollis*, 3: *H. immaculatus*, 4: *H. laminatus*, 5: *H. lineaticollis*, 6: *H. mucronatus*, 7: *H. obliquus*, 8: *H. ruficollis*.

#### *Associations between male and female characters*

There were little significant covariances found between males and female characters in this study. There were no significant associations found between the following characters: the foreleg suckers and pronotal pores (Spearman's correlation coefficient:  $r = 0.595$ ,  $P = 0.120$ ), mid-leg suckers and elytral pores (Spearman's correlation coefficient:  $r = -0.006$ ,  $P = 0.987$ ), Total number of male suckers and total density of female pores (Spearman's correlation coefficient:  $r = -0.030$ ,  $P = 0.934$ ). No significant correlation was found between large pores

on the pronotum and foreleg suckers (Spearman's correlation coefficient:  $r = -0.213$ ,  $P = 0.555$ ).

## Discussion

### Association between males and females

The present study documented the morphology of the female dorsal surface and male suckers in light of evidence for sexual conflict for the first time. Correlations of male and female traits indicated that there was a weak associations between males and females traits in the family as a whole. However there were significant differences between species of the same sex in these traits which could suggest that certain clades were evolving structural modifications independently. Observation of the histograms in Fig. 23-24 and Fig. 28-29 suggest that *H. flavicollis*, *H. ruficollis* and *H. obliquus* females have high pore densities and males and numerous suckers. *H. flavicollis* (Fig. 8-9) females evidently have more pores than males, not only more large pores but they have the addition of small pores which are absent in males. *H. ruficollis* (Fig. 20-21) females have a significant density of small pores on the elytra, which are again absent in males. *H. obliquus* (Fig. 18-19) males and females both have high pore densities and numerous suckers. Due to small sample size in this species no comparison to other male dorsal surfaces of *H. obliquus* could be made. Therefore, it is important to include other specimens of this species in future studies to verify the morphology of the male dorsal surface. Then further conclusions can be made about differences and possible covariance of characters in this species.

### Female dorsal sculpture

There were no strong associations found between sexes in Haliplidae as a family, as seen in Dytiscidae (Inoda *et al*, 2012A). There were however, significant differences found between species in female dorsal sculpture. These features were characterised by differences in pore density, type (large, medium and small) and diameter. The dorsal surface in most females appeared rough and grainy with variations of the different types pores, which were determined by size. Variation in pore density and size could occur for a number of reasons. Firstly, increases in female pore densities could be evolving in certain species as counteradaptations to conflict over mating. This is where male suckers reduce female resistance, thereby reducing the females choice over mating. There are often induced mating



costs that hinder female fitness (Arnqvist, 1997; Arnqvist, 1989). Females with a higher pore density might be able resist matings from males, as increased pores may reduce male sucker adherence to the female dorsal surface. It is important to note that not all sexually dimorphic traits occur as a result of sexual conflict. However, when considering the data in this study one can infer that there are some possible associations amongst certain species. Further experimentation would be required to test this association as results from this study are indications of possible evolutionary effects in species.

Females were found to have a significantly higher density of pores on the elytra (Fig. 24), particularly small pores on the elytral disc (Fig. 25). Small pores may create a rough, grainy surface that reduces adherence and suction from the suckers. *B. elevatus* was not statistically analysed in these analyses, however observations of Fig. 25 indicate that this species has a high number of small elytral pores. It would be interesting in future studies to further analyse this species as it had a high density of pronotal pores as well. It would be expected that large pores have the greatest contribution to reducing adherence of male suckers, as they create larger crevices and pits. Yet, on the elytra the smaller pores dominate in density. The small pores could have evolved as a counteradaptation that reduces male prehensibility by creating a rough-grainy dorsal surface. Sexual selection theory predicts that selection pressures, such as costly matings can drive the evolution of sex-related structures that drive sexual conflict between sexes (Andersson *et al*, 2000). Female counteradaptations like resistant pores have been documented in water strider females, where they have evolved modified spines to reduce the males grasping ability (Arnqvist & Rowe, 1995). The resistant ability of the different sized pores is unknown and further study would be required to clarify relative pore size and their resistance.

It has been described that in the beginning stages of copulation males approach females and grasp them using their fore and mid-legs (Vondel, 2005). However, it is not known whether males use their forelegs to grasp the pronotum and the mid-legs to grasp the elytra. The high density of small pores on the elytra reported this study may indicate that males predominately grasp the elytral disc during mating. This may result in females evolving an adaptation to contest the males trait, which could lead to unwanted and antagonistic matings. Coevolution of traits like this can lead to escalating reciprocal arms races. Behavioural observations could be employed in future studies to determine the grasping point on the female dorsal surface.

Phenotypic polymorphisms have been documented in many cases where females evolve morphologies in response to a male adaptation. This drives the evolution of coadaptations in relation to costs related to female fitness. Theoretical models suggest that antagonistic coevolutionary arms races can lead to reproductive isolation and speciation or female polymorphism's (Svensson, 2007; Gosden & Svensson, 2007). Future research into the sexual dimorphism between sexes in Haliplidae is paramount in understanding this evolutionary process.

One suggestion for the differences in female dorsal sculpture observed in *H. flavicollis*, *H. ruficollis* and *H. obliquus* could be that females are evolving higher pore densities as a counteradaptation to male secondary structures. The overall correlation analysis did not suggest covariance between male and female characters. However, there is evidence of associations between traits in the above species. Phylogenetic effects may be occurring in specific clades of Haliplidae, where selection for secondary sexual characters is more predominant than in other clades. This could be due to differences in evolutionary histories and ground body plans. Investigation of sexual conflict in discrete clades would be an important part of future study, as equivalent comparison can be made between sexes in the same clade. For instance, clades in *Brychius* could be examined separately as this genus differs significantly in morphological characteristics. Drotz *et al.*, (2010) examined the elytral reticulation in *Agabus bipustulatus*, they investigated several named forms in the *A. bipustulatus* complex and discovered several supported clades within the complex. The different clades differed in reticulation patterns and there was variation amongst male form. Discrete study into clades would provide distinct data where associated male-female characters could be examined equivalently.

### **Male tarsal modifications**

There were significant differences in male tarsal modifications, which were characterised by disparities in the number and diameter of suckers. Some species (*H. flavicollis*, *H. ruficollis* and *H. obliquus*) show potential for covariance of male-female traits. Again, this suggests that the effects occurring may be phylogenetic. It would be expected (under sexual conflict theory) that the presence of suckers on the male tarsi aid adhesion to females during mating. Male tarsal suckers have been reported as vital structures in ensuring adhesion to the female pre-

and during copulation (Aiken & Khan, 1992; Bilton *et al*, 2008; Drotz *et al*, 2010). If the female is able to resist mating, selection may act upon males to produce better suckers to grasp the female. In the absence of suckers the males reproductive success will be reduced. Despite the lack of evidence in the correlation of Haliplidae as a whole, the data suggests that there is differentiation in sex-specific traits amongst certain species. Males with a higher number of suckers would be at a competitive advantage to males with fewer suckers. Increased number of suckers would improve grasping ability on the female dorsal surface, particularly in a situation where an opposing male might try to interfere a mating (Bilton *et al*, 2008; Perry & Rowe, 2012). It would then be expected that females evolve counteradaptations to appose male adaptations, in turn sexes evolve coadaptations in a reciprocal evolution of traits (Arbuthnott *et al*, 2013).

There was a difference found in the width of suckers (width of the plate on the tip of the shaft) in males, there are possible advantages for both small and large suckers. The larger plates would be expected to grasp a larger area on the dorsal surface of the female, we would expect that larger plates would give the beetle better prehensility. Nonetheless, there could be an advantage in smaller plates, as they are better able to fit between pores. For instance, *H. obliquus* has a high pore density in females with all three pore sizes, the males possess a numerous suckers with a smaller plate width. The smaller sized plates could be adapted to fit between the different sized pores to improve prehensility. This might suggest males and females coevolving adaptations, not only in producing more pores and suckers but males are coevolving to overcome the females counteradaptation. This arms race could be defined as a reciprocal and cyclical evolution between sexes.

Haliplidae males were found to possess one type of sucker, which are relatively small suction cups (plate) on the tip of a stork-like appendage (shaft). The smaller suckers present in males are suggested to have a primary locomotory function in other beetles (Dytiscidae), as they are found in both sexes (Bilton *et al*, 2008). However, in Haliplidae the suckers are completely absent in females, suggesting they have a sex-specific function that is strongly related to aiding prehensility during reproduction (Bilton *et al*, 2008; Inoda *et al*, 2012B; Green *et al*, 2013). It is likely that smaller suckers aid locomotion and prehensility during mating (Voigt *et al*, 2008). Alternatively, certain species, as a result of their evolutionary history may have evolved secondary structures due to environmental stochasticity as variation in ecological

conditions can drive the evolution and adaptation of mating strategies (Rowe *et al*, 1994; Weir *et al*, 2011). Some species may not have been exposed to particular evolutionary pressures, therefore selection increases for sex-specific traits may not have occurred at all or they may have occurred at a lower rate. Resulting in some species with fewer suckers and reduced pore density.

Previous studies have highlighted the fact that the process of sexual conflict and arms races is often driven by highly antagonistic mating processes, for instance male harm and female resistance in *Drosophila melanogaster* (Brommer *et al*, 2012; Arbuthnott *et al*, 2013). As highlighted in the introduction, both sexes in *H. memnonius* possess small suckers. However, only the males have evolved the larger imposed suckers which have a primary sex-specific function. If large imposed suckers would provide an evolutionary advantage for haliplid males, the likelihood of selection for this trait would be high, given that larger suckers have evolved independently in other Coleoptera (Bergsten *et al*, 2001). From a genetic and developmental point of view, it is probable that haliplids would be able to produce large suckers, considering they have already produced a form of sucker. This may indicate that sexual conflict, if present in this family, is not intensively antagonistic as smaller suckers are sufficient in maintaining a firm grasp of females during copulation.

Green *et al*, (2013) make an informed point on the relative size of suckers in diving beetles. They suggest that the small suckers have greater adhesion to uneven, rough surfaces than the larger imposed suckers. This was represented in a study of the Colorado potato beetle (*Leptinotarsa decemlineata*) by Voigt *et al*, (2008). Evidence from this study suggested that the smaller suckers found in this beetle are better adapted for adhering to uneven surfaces. Whereas large suckers, as seen in dytiscids could be an adaptation to both smooth and rough (Green *et al*, 2013). One other possibility that explains the lack of larger suckers in Haliplidae could be that the relative sucker size could be linked to body size. In Syrphid flies the setal tip area increases with an increased body size (Gorb, 2001). Haliplid suckers are relatively small which may coincide with their small body size. In comparison to the larger beetles like Dytiscidae whom have increasingly larger suckers. Large suckers may not prove an evolutionary advantage for the smaller aquatic Adephaga such as Haliplidae as the suckers are relative to their body size. The larger the body size, the larger the suckers needed to maintain

suction as the force of the beetles own body adds to the resistance.

### **Mating behaviour**

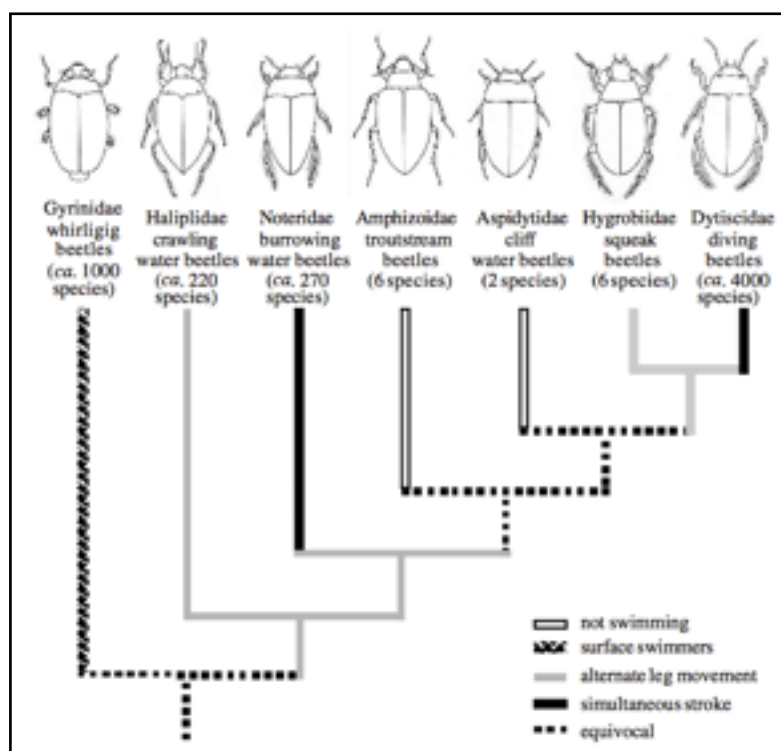
Returning to the idea of antagonistic copulations is important as it may be a key driver in the evolution of female polymorphisms (Gosden & Svensson, 2007) and in explaining the differences in male and female morphology. Sexual conflict can be driven by long copulatory durations and females often perform pre-copulatory and post-copulatory struggles to resist copulations and to reduce post-copulatory guarding time (Bergsten *et al*, 2001; Benvenuto & Weeks, 2012). In Dytiscidae, sexual conflict is driven by such factors and mate guarding can last from 5-10 hours in some species (*Dytiscus alaskanus*). (Bilton *et al*, 2008). Long pairing durations often increase fitness costs for females. For instance, dytiscids mate whilst submerged underwater, periodically surfacing to renew their air supply. However, when the male surfaces for air he is still mounted to the female, which limits the amount of air she can renew. Physiological costs like this have a negative effect on female fitness, which drive the evolution of counteradaptations to contest detrimental male secondary structures. In this family females have evolved micro reticulation to increase resistance during copulation and improve detachment ability. In Haliplidae, copulatory durations are much shorter and females do not possess a great deal of force to vigorously shake off or detach the male.

The differences in copulation duration between these two families mirrors the extent of sexual conflict that may occur. The longer the copulatory duration lasts the greater the fitness costs incurred by the female. Thus, the greater the arms races between the sexes as females evolve counteradaptations to contest the associated costs. Although there is a relatively short copulatory duration in Haliplidae, this does not mean that there are no fitness costs acquired at all, minimised costs can still effect mating behaviour and female adaptations. For instance, in gerrids the pairing duration is short (minutes to a few hours) yet the costs acquired by females have driven the evolution of counteradaptations between the sexes (Arnqvist, 1997; Gagnon & Turgeon 2011).

### **Swimming behaviour**

The evolution of swimming behaviour can have a considerable effect on copulation processes (Ribera *et al*, 2002). There are a large number of aquatic Coleoptera that have modifications of the male tarsi - some are more extreme than others (Fig. 32). When a beetle has

simultaneous stroke during swimming it increases the velocity that propels the body through the water, resulting in higher swimming performance (Balke *et al*, 2005; Ribera & Foster, 1997).



**Figure 32.** The evolution of swimming behaviour in the aquatic families of Hydradeephaga that may be associated with morphological traits. Note Dytiscidae and Noteridae have well developed hind legs for simultaneous stroke. Whereas, Haliplidae have long slender hind legs with alternate leg movement. Figure taken from Ribera *et al*, 2002.

In beetles where there is simultaneous stroke, females have more velocity to propel their powerful hind legs that are better at dislodging males and drive the beetle through the water, increasing female resistance. This causes antagonism during copulation as there often a struggle between males and females. This, in turn, is more likely to create extreme trade-offs during copulation as each sex evolves adaptations to contest the adaptation of the opposite sex, which results in a coevolution of traits. It is possible to suggest that large suckers, as seen in Dytiscidae, might be present in groups due to simultaneous stroke. Whereas, groups like Haliplidae have alternate stroke there is often a slower less efficient swimming pace. Reducing conflict during copulation as females have less power to detach males and swim

away, this may minimise the need for strong secondary structures like large suckers in males (Ribera *et al*, 2002).

In families where there is simultaneous stroke, and females have powerful hind legs for swimming, the males may have evolved larger suckers for increased suction strength to maintain a grasp on the females dorsal surface while she attempts to swim away. If haliplid females have less strength to resist males and swim away (due to alternate stroke) then selection for larger suckers would be reduced, as they have a lower evolutionary advantage because the smaller suckers are suitably prehensile. It has also been suggested that smaller suckers are better adapted to adhering to uneven surfaces (Voigt *et al*, 2008), such as increased pore density. Increased antagonism in Dytiscidae that is weak in Haliplidae that drives the escalating evolution of adaptations and counteradaptations of sexual characters. Copulation behaviours and adaptations for resistance vary greatly and are subject to a wide range of influential factors, therefore the intensity of conflict can vary greatly (Arnqvist, 1997). If large suckers do not provide an evolutionary advantage to males then it is unlikely they would be selected for. This behaviour could determine the strength of the evidence provided for sexual conflict in Haliplidae as a family. It is highly probable that species in certain clades may have stronger stroke than others, evidence of this is required. This further supports the notion that the evolution of antagonistic copulations could be developing in clades rather than in the family as a whole.

### **Directions for future research**

This area of evolutionary biology merits further study as this kind of behaviour has evolved independently within Dytiscidae, rather than within the aquatic Adephaga as a whole. Despite there being no association between male and female traits as a family, there are significant differences in the dorsal surface in females and within various sucker characteristics in males. A more focused analysis exploring the differences in morphology within a particular clade of haliplid where we know the interrelationships between species would be appropriate step for further study. By examining certain clades we are able to compare like with like traits as there are specific ground plans for sculpture. Each clade will have a particular dorsal surface in females and tarsal modifications in males, which may differ from another clades.



Research into the of the adhesive potential of the male suckers would be beneficial to the understanding covariance between the male suckers and female pores as the adherence of the suckers against different sized pores is unknown. Aiken and Khan (1992) investigated the adhesive potential of the suckers of the male boreal water beetle, *Dytiscus alaskanus*. They tested the mass that the large primary and secondary suckers were able to lift. Evidence from this study inferred that large suckers accounted for 59% of the adhesive strength and suckers were able to hold up to four times the weight of a female. Stork (1980) examined the male tarsal setae of *Chrysolina polita* and reported that the adhesive force increased with the number of adhesive setae. Han *et al* (2010) similarly investigated grasping force in male gerrids and found that female resistance was significantly reduced with increased male force, future research in this are would be valuable.

Direct evidence is needed on the effect of copulation on female fitness, this will advance the understanding of copulation behaviour of Haliplidae. There are many studies that provide examples of sexual conflict, but there are a few that have assessed the economy and costs of sexual traits (Fricke *et al*, 2009). Pairing duration and copulation durations should be studied and the relative costs analysed. Understanding the exact fitness costs associated with copulation in females is vital in the understanding sexual conflict in this family (Miller, 2003).

Density and environmental factors should be taken into consideration when examining copulation behaviours. Previously, such effects have been somewhat neglected during investigations (Fricke *et al*, 2009). Recent data have established the economics of sexual conflict between sexes and how the environment and condition of mates can largely affect the development of sexually antagonistic coevolution (Miller, 2003; Kokko & Rankin, 2006; Candolin & Heuschele, 2008). Variations in ecological factors can determine the costs and benefits for each sex and sex ratios, and population densities have been shown to effect male mating behaviour (Rowe *et al*, 1994; Rundle *et al*, 2000). Future research into the ecological factors that affect mating systems would advance knowledge of the extent at which sexual conflict can occur.

## Conclusions

This initial investigation provides insight into the differentiation in male and female sculpture.

I have demonstrated that females differ between species in dorsal sculpture and males differ between species in tarsal modifications. Although there was no association of male and female characters overall, these differences in male and female specific character indicate that there may be covariance in certain haliplid clades. Sexual conflict can drive the adaptation of secondary structures in males but also the coevolution of morphological adaptations in females, resulting in sexual dimorphism and coevolutionary arms races between sexes. Understanding these processes is important on a wider scale as the costs of copulation directly and indirectly affect life history traits, such as lifetime fecundity and survival, both of which are major determinants of population dynamics (Wedell *et al*, 2006). The present study provides a valuable insight into sexual dimorphism in Haliplidae and provides possible causes for the differentiation in morphology between the sexes. Evidence from this study suggests that economics over mating may be a key driver of sexual conflict which may be occurring in specific clades of Haliplidae. As a result, further investigation into independent clades should commence, based of the information gained from this study. More importantly, future research can enhance knowledge on the process of sexual conflict as can driver evolutionary change (Gavrilets 2000; Tregenza *et al*, 2005).

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